NIH Korea National Institute of Health

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Message from the Director General

"Promoting Public Health and Quality of Life"

Korea National Institute of Health (KNIH) conducts and supports biomedical sciences and health research to improve public health and quality of life. KNIH supports national agencies to develop scientific evidence-based government policies for promoting public health, and also provides national infrastructures of biomedical sciences with research institutions and health care providers. KNIH has shared visions "Global Leader Pioneering Innovative Health Research" with an emphasis on core values of "Openness, Collaboration, Challenges, and Future". In recent years, our remarkable achievements have been made in several major areas of infectious diseases (tuberculosis, influenza, AIDS, etc.), non-communicable diseases (diabetes, cardio-cerebrovascular diseases, metabolic diseases, Alzheimer's disease, etc.) and genome science (genetic risk factors for chronic complex diseases) as well as infrastructural sciences (National Biobank of Korea, National Center for Medical Information and Knowledge, National Center for Stem Cell and Regenerative Medicine, etc.).

This is the third year of relocation of KNIH in Osong where we opened the new Osong bio-health era in 2011. The KNIH campus housed in the Osong Health and Technology Administration Complex that has clearly become one of the premiers research arenas in Korea. During the past three years in Osong, we have advanced public health sciences largely in the context of infrastructural sciences, and expanded national and international research collaborations and networks. Especially, by rapid testing in the laboratory diagnosis of emerging pandemic pathogens for the past year in 2013, we have successfully prepared for threats of emerging and re-emerging

infectious diseases including SFTS (Severe Fever with Thrombocytopenia Syndrome), MERS (Middle East Respiratory Syndrome), and China's H7N9 influenza. We were also successful in completion of constructing a new facility of the National Center for Medical Information and Knowledge which will provide the knowledge-based research engine to promote biomedical sciences and public health research.

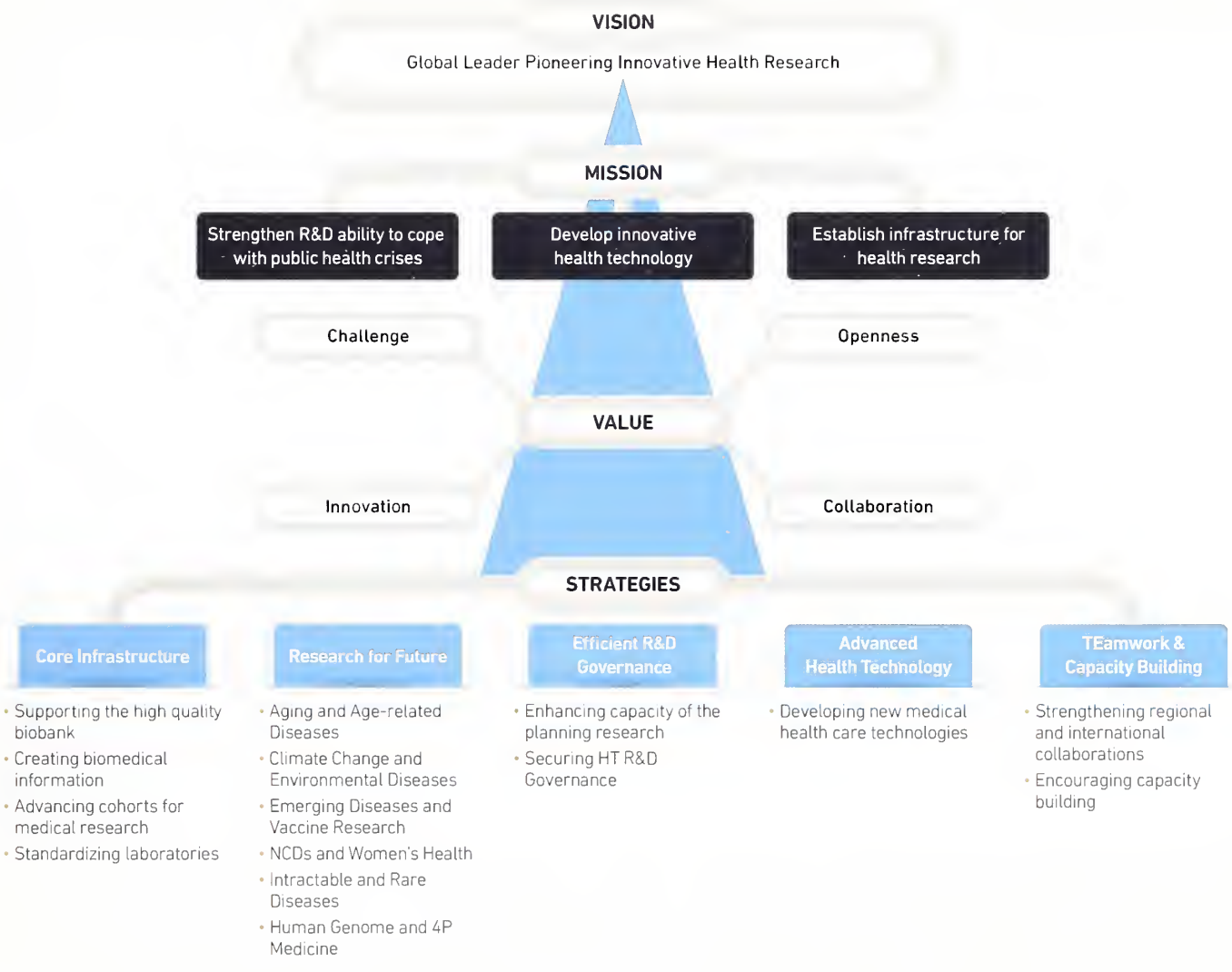
KNIH has now grown to nearly 500 people including enterprising government staffs and enthusiastic scientists, who think big and innovatively, tireless working to advance public health and ultimately envision the world free of diseases with the KNIH vision "Global Leader Pioneering Innovative Health Research". In the coming years, you will see KNIH playing a leading role in advancing biomedical sciences and public health promotion in Korea. I encourage you to explore this Annual Report outlining the many impressive achievements of the KNIH.

Lee Joo-Shil, Ph.D
Director General, Korea National Institute of Health

Vision & Mission

A Global Leader Pioneering Innovative Health Research

Korea National Institute of Health reconstructed its vision and strategy, and established the long-term plan according to the national agenda and environmental changes in order to make a new leap forward disease-free world on October 2012. 'VISION of KNIH' sets 5 strategies and 10 initiatives to achieve the goals.



History

Providing scientific evidences for the public health and reducing the burden of diseases..... **KNIH**

KNIH was established in 1945 to promote public health via disease-related research and development. As a part of the Ministry of Health and Welfare, KNIH plays a key role to illustrate the causes of various diseases and develop effective measures for the diagnosis, prevention and therapy of communicable and non-communicable diseases. It has also contributed to establishing state-of-the-art infrastructures and striving for the ongoing progress by providing scientific evidences for the public health and reducing the burden of diseases.

1945	The Korean Government established the Jo-Seon Quarantine Laboratory and National Chemical Laboratory.
1963	KNIH was combined with National Quarantine Laboratory, National Chemical Laboratory, National Institute of Health and National Biological Medicine Testing Center.
1970s	Viruses such as Rubella and Mumps were categorized by the diagnosis system. WHO designated the National Influenza Center in KNIH.
1980s	KNIH developed and investigated unknown diseases such as legionellosis (1984), leptospirosis (1984), AIDS (1985), *Vibrio vulnificus* sepsis (1986), etc.
1990s	A Special Disease Department, currently Center for Biomedical Sciences, was created to introduce gene text technique and develop national diagnosis system.
2000s	The center for Genome Science was established(2001). KNIH renamed the Korea National Institute of Health(2004).
2011	KNIH was relocated in Osong, Chungbuk to become a global biomedical research complex.
2012	The National Biobank of Korea was completed to conduct biospecimen research and secure Korean Cohorts. The Division for Vaccine research was set up for preventing and treating infections with advanced research technology.
2014	KNIH established the National Center for Medical Information and Knowledge.

Organization

NIH

Of the **KNIH**
By the **Research**
For the **Public health**

TF: Pathogen resource TF, Allergy TF, Clinical Research Coordination TF, National Center for Medical Science Information TF, BioResources Management of Health Medicine TF,Medical Testing Accreditation TF, Laboratory Support TF, International Collaboration TF, Medical Science Lab StandardizationTF, Korea BiobankProject TF, BiospecimenDistribution TF

Facilities

Biomedical Sciences and Genome Science

Conducting stem cell and a regenerative medicine research aimed at overcoming the incurable diseases

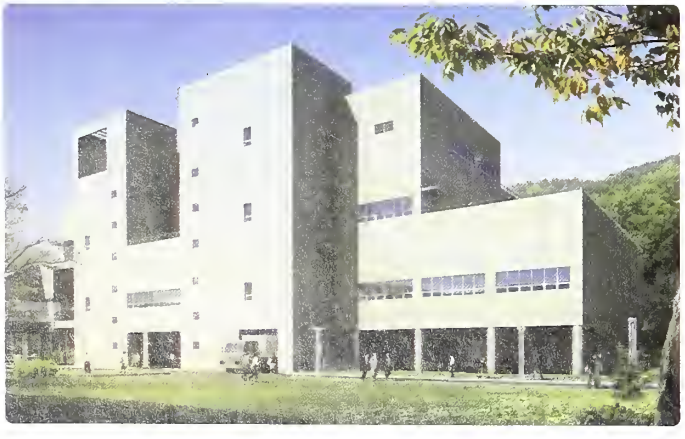
Creating biomedical resources and infrastructure for advanced medical research and technology

Infectious diseases and Immunology & Pathology

Enhancing the national response capacity on infectious diseases

Carrying actively out the human health adaptation research to the climate change affected by the environment

Preparing for the research on newly emerging infectious diseases due to changes of lifestyle and ageing



Biosafety Level 3 Laborotory & Animal Facilities

Inspecting emergence and reemergence of pathogens and enhancing the biosafety capacity of buildings regarding the national certification and management of high-containment facilities

Providing for the proper care and maintenance of all laboratory animals for supporting to animal studies

National Biobank of Korea

Collecting, managing and distributing population-based biospecimens

Conducting the Korea Biobank Network with 17 regional biobanks at university-affiliated hospitals

Improving sample quality and developing biobanking tools

National Center for Medical Information and Knowledge

Collecting, integrating, disseminating knowledge and providing medical science information at a national level

Completion in 2014

National Center for Stem Cell and Regenerative Medicine

Developing personalized medicine using stem cell

Conducting translational and clinical researches

Promoting an international standardization

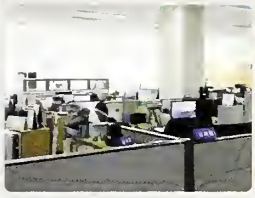
Completion in 2015

National Biobank of Korea(NBK)

Storage Facilities

Laboratory

Offices



Reception rooms of human biospecimens

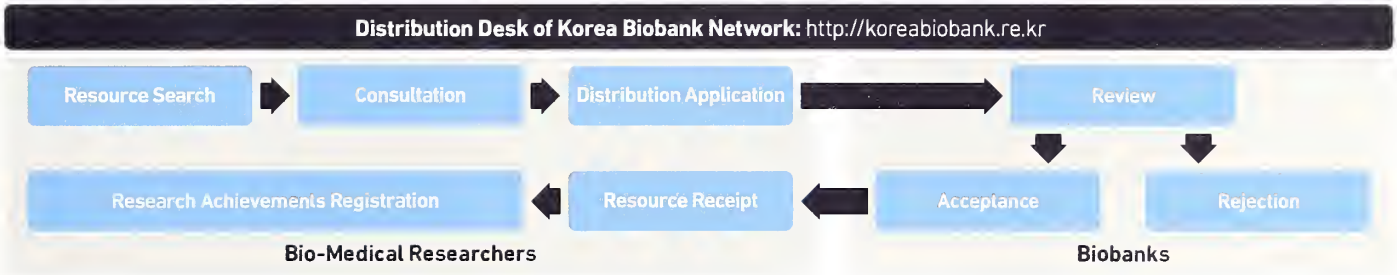
Conference & Meeting rooms

Roles

Collecting, managing, and distributing population-based biospecimens
Conducting the Korea Biobank Network with 17 regional biobanks at university-affiliated hospitals
Studying on Improvement of sample quality and biobank management

Online Distribution System

The Korea Biobank Network developed an online distribution system entitled "Distribution Desk of Korea Biobank Network(http://koreabiobank.re.kr)". Using this system, bio-medical researchers can search information of human biospecimens, consult consultant in the National Biobank of Korea about distribution of human biospecimens and related information, and apply distribution.



[Distribution of bio-resources through the Distribution Desk of Korea Biobank Network]

National Center for Medical Information and Knowledge(NCMIK)

MCMIK collects and provides research information such as papers dissertations, magazines, journals as well as electronic materials. Users can directly access all materials. The library provides user-friendly environments for those who are in the fields of public health. The NCMIK has an on-site book cafe accommodating 150 users in the 1st floor.

Visitors Policy

If users join the website(**http://library.go.kr**), more services can be provided.

Operation Hours

Monday - Friday : 09:00 ~ 20:00

After 18:00, users can use only the book cafe(1F) and Reference Room(2F).

CLOSED on Saturday, all holidays and days determined by the library

Process

Joining a membership → Issuing an ID card → Passing a main gate → Using the library → Leaving the room

Application of Tour

Personal or group visit is available by application on the website.

Services

Computers are provided in every floor.

- Special and Cultural Books
  - Journals, books, etc., on biomedical research
- Reports
  - Reports issued by national institution, policy research institute, etc.
- Journals
  - Journals and magazines regularly issued by special agency in Korea
- Electronic resources
  - Searching, reading or printing original texts of DB or journals
  - 10,000 E-journals, 160,000 E-book, 20 WebDB

Guide of using facilities

1F

Book Cafe

- Newsletter, newspapers, etc.

Gallery

- History, achievements of KNIH and KCDC, special exhibitions, etc.

Special Education Room

- Group training operated in the room (44 seats).
- Users can use the room with advance application. (Contact: 82-43-249-3029)

2F

Reference Room

- Specialized books, reports, journals, on biomedical research

Personal Research Room

- Reservation is required for personal research in advance.

3F

Multimedia Room

- Web DB, searching original document, digital materials, DVD, audio, etc.

National Culture Collection for Pathogens (NCCP)

NCCP has been established as the sole national pathogen resource bank in Korea in order to promote biomedical and public health sciences and provide researchers with various high-quality clinical pathogens isolated and standardized from Korean clinical patients.

- Collecting, preserving and managing clinical isolates for pathogen resources in Korea
- Searching and discovering emerging and re-emerging pathogens
- Developing resources, aiming for the development of diagnoses and vaccines, etc.
- Operating a deposit system of R&D achievements
- Promoting international resource exchange

Distribution of pathogen resources

NCCP manages and distributes pathogen resources for researchers in the fields of academic, institutional and industrial fields.

- Pathogens isolated from clinical patients
- High risk pathogens
- Pathogens with the specific information (serotype, genetic type, antimicrobial resistance and clinical information, etc.)
- Derivatives (Nucleic acid, antigens, antibodies and biomarkers)

* Researchers in any institute with proper lab facilities with regard to biosafety level, can order pathogens according to the guideline.

ACCESS AND DISTRIBUTION

01 Search and inquire for accessible resources
- log in website (http://nccp/cdc.go.kr)
- Direct contact (82-43-719-6670)

02 Submission of request forms
- Official document for request
- Research proposal
- Access request from
- Lab or facility photo

e-mail : nccpbank@gmail.com
Fax: 82-43-719-6680

03 Access review
- Reviewing a request within 10 days
- Distributing resources within 30 days after access approval

04 Resource receipt and feedback
- Official document for receipt
- Receipt with a revenue stamp

International Collaborations

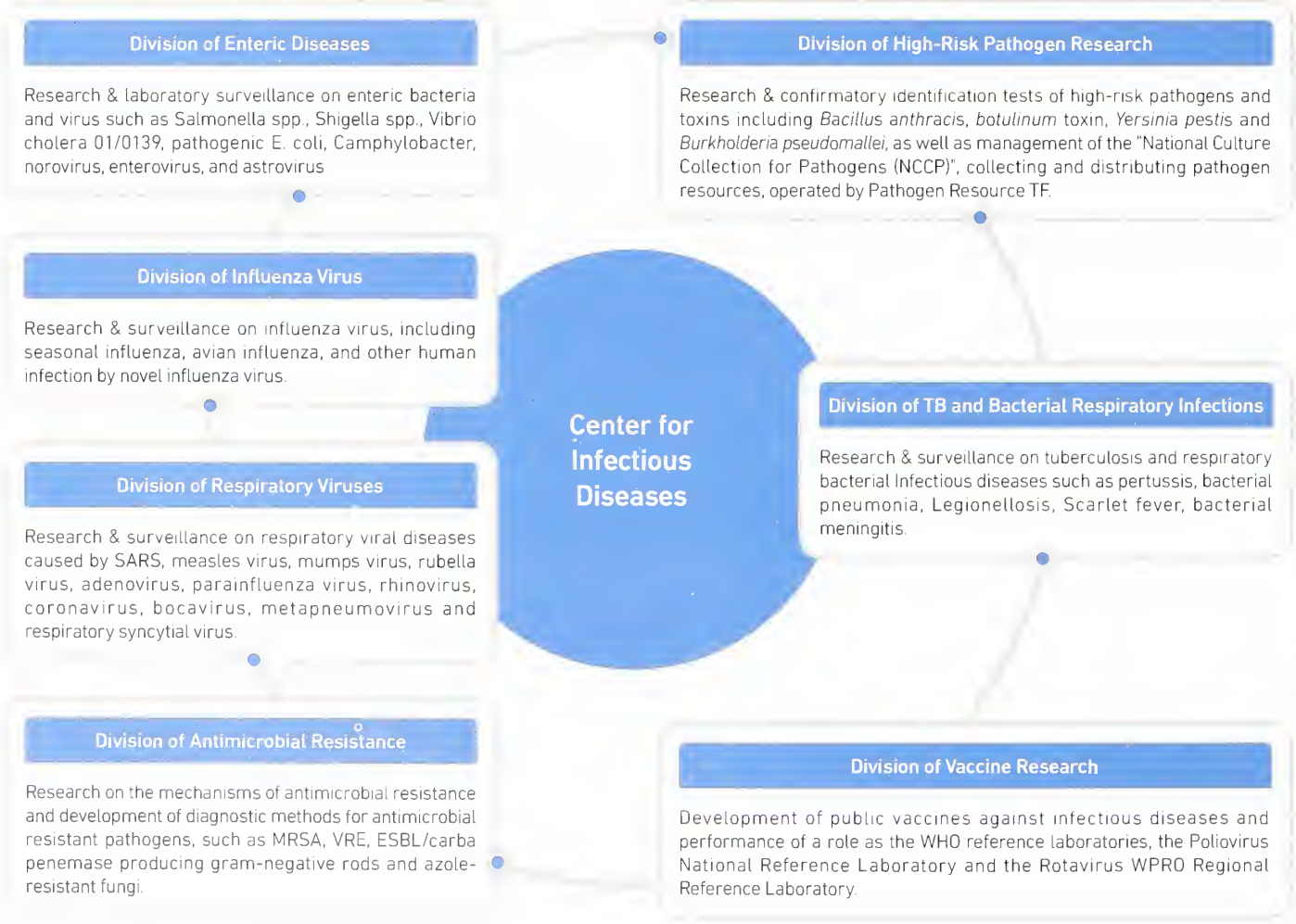
COLLABORATION INSTITUTE

KNIH has made agreements with many public health research institutions around the world to enhance and expand international cooperation in health and medical sciences. KNIH also supports research environments of developing countries by official development assistance for improving public health. KNIH has established domestic collaborations with various organizations including research institutes, universities and hospitals across the country for cooperative exchanges of biomedical resources and knowledge.

NATIONS	COLLABORATION INSTITUTES
 **USA**	National Institutes of Health
	National Institute on Alcohol Abuse and Alcoholism(NIAAA)
	National Institute of Child Health and Human Development(NICHD)
	National Institute of Allergy and Infectious Diseases(NIAID)
	Harvard School of Public Health
 **UK**	National CJD Research &Surveillance Unit(NCJDRSU)
	National Institute for Biological Standards and Control(NIBSC)
 **Germany**	Leibniz Institut DSMZ - German Collection of Microorganisms and Cell Cultures
	Berlin-Brandenburg Center for Regenerative Therapies
 **China**	Yanbian Center for Disease Control and Prevention
 **Japan**	National Institute of Biomedical Innovation (NIBIO)
 **Vietnam**	National Institute of Hygiene and Epidemiology
 **Mongolia**	National Center for Communicable Diseases
	Institute of Veterinary Medicine
 **Bhutan**	JigmeDorjiWangchuck National Referral Hospital
 **Israel**	Tel-Aviv University
	International Vaccine Institute
	Institut Pasteur Korea

Center for Infectious Diseases

The Center for Infectious Diseases (CID) conducts and supports various intramural and extramural researches to better understand, treat, and prevent infectious diseases: enteric pathogens, influenza, respiratory viruses, antimicrobial resistance, high risk pathogens, TB and bacterial respiratory infections, and vaccine development. A variety of resources from the researches have been served to the related researchers by NCCP (National Culture Collection for Pathogens) and DB. In order to enforce the laboratory surveillance for infectious diseases, the cooperative system between laboratories of 17 province environmental and health institutes and CID has been operated through financial and technical supports. In addition, the laboratory response network has been maintained to monitor the bio-terror agents. The WHO collaborative laboratories for Infectious Diseases have been maintained to strengthen the international networks (WHO/WPRO Regional Reference Laboratory for invasive bacterial vaccine preventable diseases, WHO poliovirus national reference laboratory, WHO national influenza center, and WHO national measles laboratory).



We will make a continuing effort to develop the improved and new diagnostics, treatment, and vaccines to support control policies for the emerging and re-emerging agents as well as major infectious diseases.

Research on Infectious Diseases

ENTERIC PATHOGENS

A nationwide inverstigation was carried out to determine the prevalence of the types of extended-spectrum beta-lactamases (ESBLs) produced by *Salmonell*a Enteritidis.

A total of 1,087 clinical strains from 2008 to 2012 were screened for resistance to extended-spectrum cephalosporins. Polymerase chain reaction (PCR) and sequencing were used for analysis of *bl*a genes, and the plasmids were transferred using the broth culture conjugation method. Standard methods were used for clonal (pulsed-field gel electrophoresis [PFGE] and multiple-locus variable number tandem repeat analysis [MLVA]) and plasmid (replicon typing and plasmid double locus sequence typing [pDLST]) analysis. A total of 140 *S.* Enteritidis isolates were ESBL positive by their phenotype. A nucleotide sequence analysis of the 140 isolates carrying genes encoding ESBLs revealed that they harbored $bla_{CTX\text{-}M\text{-}15}$(n=138) and $bla_{CTX\text{-}M\text{-}55}$ (n=2), which were transformable. Plasmids carrying $bla_{CTX\text{-}M}$ genes were assigned to IncHI2 (n=120), IncFI (n=8), or IncI1 (n=12) using a PCR-based replicon typing method. All of the IncHI2 plasmids carrying $bla_{CTX\text{-}M\text{-}15}$ were ST2 types according to pDLST. Seventeen PFGE-*Xba*I patterns and four MLVA patterns were identified. The isolates revealed highly similar PFGE/MLVA patterns indicating clonal relatedness across different regions and years. From these results, we found that the common type of ESBL in *S.* Enteritis after 2008, in Korea, was the CTX-M type. Most ESBLs were encoded by transformable plasmids and the majority of transformable plasmids were IncHI2-ST2 types. The spread of $bla_{CTX\text{-}M\text{-}15}$ was attributed to clonal expansion and to horizontal dissemination of related plasmids.

In the enterotoxigenic *Escherichi*a co*li* (ETEC) H10407 the *lt*-A and *msbB* genes were inactivated strain to test detoxified ETEC-mOMV as a vaccine, and the resulting Δ*lt*-A/Δ*msbB* mutant was used as the detoxified ETEC-mOMV producer. After characterizing the mOMVs, they were utilized in prime-booster vaccine experiments to evaluate the immunogenic potential of the less-endotoxic mOMV lacking LT-A. In addition, purified SigA2, which is a 294-amino acid fragment of SigA was mixed with ETEC-mOMV to produce a subunit vaccine against *S. flexneri* 2a to determine whether ETEC-mOMV exerts an adjuvant effect on the SigA2 recombinant antigen. The mice that received the mOMV by intramuscular injection at 2-week intervals showed a high IgG antibody titer towards the outer membrane proteins of ETEC, suggesting that the mOMVs are fully immunogenic without co-administration of commercial adjuvant. Interestingly, the SigA2 subunit vaccine combined with the mOMV protected the vaccinated mice from challenge with *S. flexneri* 2a intraperitoneally. These results suggest that the detoxified ETEC-mOMV could be a safe vaccine immunogen, as well as an effective adjuvant towards a recombinant protein subunit vaccine in a mouse model.

DEVELOPMENT OF AN INFLUENZA VACCINE AND DRUG RESEARCH

Since the H1N1 pandemic in 2009 and avian flu outbreaks on poultry farms, which threatened our society and public health, the Division of Influenza Virus has implemented active studies to develop an influenza vaccine and investigate antiviral drug candidates under more efficient and rapid production processes against a future influenza pandemic through both external research projects and internal studies.

Vaccine development–related projects were undertaken by external research (development of an avian influenza vaccine) and three internal vaccine projects are being carried out in the Division of Influenza Virus.

An avian influenza A(H5N1) vaccine has been in development since 2007, and the phase III clinical trial was enrolled in 2013.

In an internal study titled "Study of hetero-subtypic immunity against influenza viruses with influenza viral products based on the hemagglutinin stalk domain (2011–2013)", HA2-based recombinants derived from the influenza virion were designed in 2011–2012 and their effectiveness as vaccine candidates was evaluated in an influenza viral infection animal model for a pan-flu vaccine in 2013.

We also elucidated the T-cell epitopes on HA, NP, and M1 antigens of the H1N1pdm09 virus in an internal study titled "Immunological characterization and epitope mapping of 2009 H1N1 influenza virus (2012–2013)".

In addition, several internal studies (3 year project: 2013–2015) for preparedness against avian flu outbreaks in humans titled "Research for rapid and safe screening of immunogens, antivirals, and virulent factors to highly pathogenic avian influenza viruses (HPAI)" was launched in 2013.

In a bundled study titled "Development of VLP-based drug screening system against highly pathogenic and novel variant influenza viral infections," an avian influenza viral VLP-based drug screening system was established in 2013.

In another study titled "Development of a rapid and efficient immunogen screening system against highly pathogenic and novel variant influenza viral infections", a virus free DNA vaccine development strategy was adopted to screen efficient vaccine antigens in 2013.

Information and research products from the projects mentioned above will be open and shared in public in 2014–2015 through research publications, pending patents, and research products. In addition, we will investigate the anti-flu drug candidates using an established VLP-based screening system with drug repurposing strategy and will establish a system to screen an efficient vaccine antigen based on a DNA vaccine strategy.

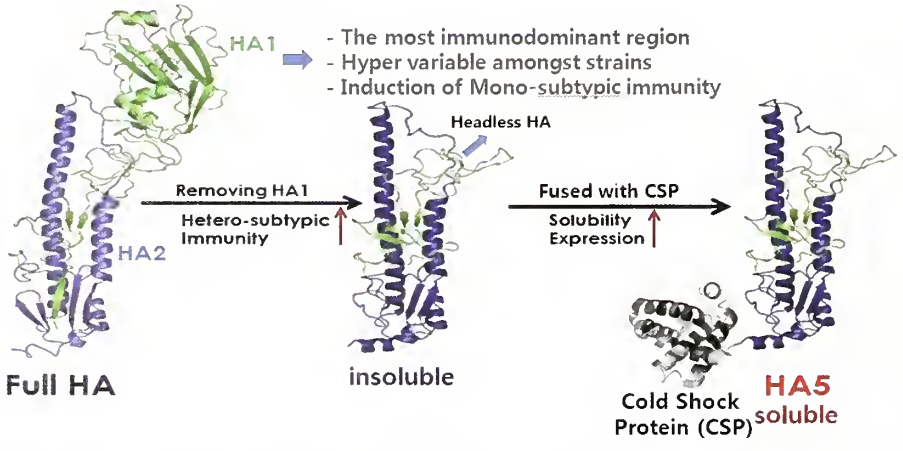


FIGURE 1. Design of a recombinant immunogen (HA5) based on the hemagglutinin stalk domain to research a universal influenza vaccine. Removing the immunodominant and hyper-variable region (HA1) in the influenza virus hemagglutinin increases the hetero-subtypic immunity with Headless HA. Adding cold shock protein to Headless HA enhances the stability and expression of the recombinant protein (HA5).

VIRAL RESPIRATORY PATHOGENS

Acute infectious disease caused by various respiratory viruses has gradually become a significant public health issue. The continuous emergence and re-emergence of respiratory viruses caused by a wide range of pathogenic changes, genetic mutations, and predicted alterations in the host immune system and/or herd immunity should be investigated continuously. The division of respiratory viruses conducted studies on the pathological importance of respiratory syncytial virus (RSV), human rhinovirus (HRV), human adenovirus (HADV), and human metapneumovirus (HMPV), as well as measles, mumps, and rubella (MMR), which will be crucial for a rapid response to current or future public health consequences.

Respiratory syncytial viruses are a common causative agent of respiratory illness. Infants < 2 years of age are prone to viral infections and re-infections occasionally throughout life. Nevertheless, no commercial vaccine is available to prevent RSV infection. Only a high cost immune therapeutic strategy is available for passive immunization. The division of respiratory viruses has addressed this issue and related vaccine development activities are being accelerated.

We reported previously in 2012 the newly emerged RSV strain ON1 with identical duplication of 72 nucleotides in the G gene. This novel RSV strain has appeared dramatically, according to population-based molecular evolutionary evidence in Korea. An evaluation of subordinate antigenic change has been performed and a research project to develop an effective vaccine candidate using the modified vaccinia vector system is underway. Our focus with RSV vaccine development is to understand RSV re-emergence and help identify clues for successful development of an RSV preventive vaccine.

Measles, caused by the measles virus (MeV), is one of the most properly controlled, vaccine preventable, diseases in the world. Although the elimination of measles was declared in Korea in 2006, sporadic outbreaks related to imported measles cases have been continuously reported. The majority of patients were infants who never received a measles vaccination and measles spread throughout the hospital. Small occasional outbreaks of measles infection in middle or high school students who had a two dose vaccination history have also been reported. The sources of infection were successfully epidemiologically and molecular biologically identified as imported or imported-related cases. The Division of Respiratory viruses is attempting to unveil why primary or secondary vaccine failure occurred among vulnerable middle or high school students using serology and virology approaches. A full sequence analysis of different genotypes of the virus was conducted to analyze genetic or antigenic drift of MeVs isolated in Korea. The plaque reduction neutralization test (PRNT) and avidity test using an IgG strategy were also used to investigate sero-protectivity of a

TABLE 1. Global or domestic public health crisis caused by respiratory viruses

Years	Events related respiratory viruses	Scale
2008-2009	Pandemic Influenza virus	Global
2009	Emergence of Human rhinovirus type C	Global
2010	Human Adenovirus Outbreak	Domestic
2011	Atypical pneumonia with unknown reason	Domestic
2011	Identification of Novel strain of RSV	Global
2012	Middle East Respiratory Syndrome CoV (MERS-CoV)	Middle East
2012-2013	Change of parainfluenza virus subtype	Domestic
2013	Outbreaks of Measles	Global
2010-2013	Mumps Outbreak	Global

specific immunoglobulin against MeV derived from successful vaccination. Data analyzed from these experiences will guide the direction of control when sporadic measles cases occur in a highly vaccinated population in developed countries.

The division of respiratory virus has also utilized a baculovirus expression system to generate matrix protein-based virus-like particles (VLPs) as a new vaccine development strategy. Several advantages are reported when using recombinant baculoviruses and insect cells to produce VLPs, including abundant production, similar post-translational modification with mammalian cells and easy co-expression of the most immunogenic viral glycoproteins. A bacuolvirus based VLP production strategy also has disadvantages, such as host protein contamination derived from insect cells or wild type baculoviruses. To circumvent these obstacles, we conducted a creative R&D project to produce a shRNA-mediated replication-deficient baculovirus/insect cell system. This system will be utilized to produce a short-cut strategy for purifying and facilitating VLP-mediated vaccine development at an industrial scale.

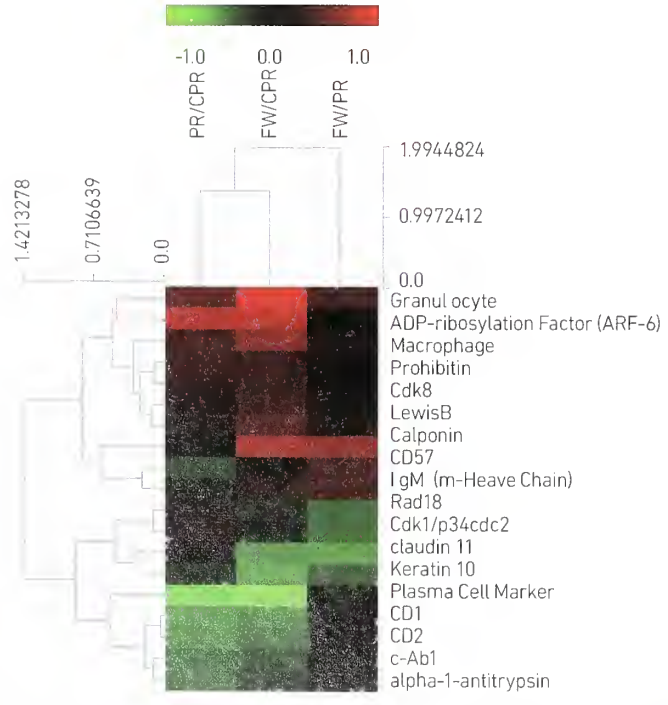


FIGURE 2. Hierarchical clustering analysis of antibodies expressed in the plasma of progressed tuberculosis and of healthy controls using antibody microarray. PR, tuberculosis progressor; FW, follow-up tuberculosis progressor; CPR, healthy controls. Gene filter: 1.5 fold, clustering program: MeV 4.7.1, clustering method: Euclidean distance, average linkage.

Continuous research activity will be conducted to establish rigid scientific evidence against public health manifestation caused by various respiratory viruses including HMPV, HRV, RSV, MeV, mumps virus, and rubella virus in the division of respiratory viruses.

TB AND BACTERIAL RESPIRATORY PATHOGENS

An infected host has a variety of clinical symptoms after they have been exposed to *M. tuberculosis*. About 10% of all infections result in active disease within 2 years. Another 10% of cases may experience disease after a latent phase over many years. The course of tuberculosis (TB) infections is determined by the way *M. tuberculosis* is removed by host innate or adaptive immunity. The detailed mechanisms of the progression of a TB infection remain unknown. Identifying a TB biomarker would improve the understanding of TB pathogenesis and diagnosis of TB infection. We attempted to identify predictive biomarkers of TB using contacts of patients with TB who later developed TB versus contacts who remained healthy, using antibody microarrays platforms. We compared antibody expression differences in whole plasma from TB progressors among contacts of TB patients, follow-up TB progressors, and healthy controls (n=10 per group). We found differential expression in 17 of 656 antibodies, and these differences in antibodies expression were validated by enzyme-linked immunosorbent assay (ELISA) and additional samples. The Rad-18 response was significantly lower in the TB progressors, as compared to that in healthy controls ($p<0.0001$). When combined, interferon gamma and Rad-18 accurately identified 93.3% of predictive TB infections. The combination of interferon gamma and Rad-18 responses was accurate for distinguishing patients with predictive tuberculosis from subjects with TB contact.

An important part of investigations into pneumococcal pathogenesis has been to describe the mechanism that pathogens use to colonize the respiratory epithelium, invade the blood stream, and cause a fatal disease. In our previous study, upregulation of genes encoding the glycosyl hydrolase family was observed in the opaque phase associated with pneumococcal invasion. Therefore, we performed a functional analysis to understand the role of glycosyl hydrolase family proteins during

pneumococcal invasive infections. We constructed knockout mutants of *S. pneumoniae* D39 lacking GH1, GH13, GH20, GH32, GH38, and GH125 and found six mutants that displayed changes in physical properties, virulence, and gene expression profiles. Compared with wild-type, six mutants displayed similar colony morphology and cell shapes but lower growth rates. In an *in vitro* virulence test, the adhesion abilities of GH1 and GH32 mutants to A549 human lung epithelial cells were decreased by up to −47% (p=0.3872) and −97% (p=0.0021), respectively. In a murine pneumonia model, six GH mutants led to attenuation in virulence. In particular, loss of GH1 and GH32 inhibited colonization in the nasal cavity, invasion to the blood stream, and multiplication of *S. pneumoniae*. A comparison of gene expression profiles between wild-type and GH mutants revealed that the GH1 and GH32 mutants upregulated gene clusters related to glycolysis, competence, stress, and capsule production by more than two-fold (p<0.05). Our results suggest the potential of glycosyl hydrolases in the control of various aspects of carbohydrate metabolism and virulence in *S. pneumoniae*.

ANTIMICROBIAL RESISTANCE AND ANTIVIRAL AGENTS

Research on antimicrobial resistance including studies on new resistance mechanisms, molecular epidemiology, and the development of target molecules for important antimicrobial resistant pathogens such as methicillin-resistant *Staphylococcus aureus* (MRSA), vancomycin-resistant Enterococci (VRE), carbapenem-resistant Enterobacteriaceae (CRE), and multi-drug resistant Gram-negative rods is necessary to identify targets and provide fundamental knowledge to develop new diagnostic methodologies, novel therapeutics, and interventions to prevent the emergence and spread of resistant pathogens in hospitals and the community. This year, we have focused on studying the resistance mechanisms and virulence factors of MRSA and vancomycin intermediate resistant *S. aureus* (VISA). Additionally, transmission & resistance mechanisms of vancomycin-resistant *Enterococcus faecium*, and carbapenem-resistant Enterobacteriaceae were studied.

ST5-SCC*mec*II (52%) and ST239-SCC*mec*III (19%) were the most prevalent among VISA strains collected from the VRSA surveillance system over 11 years (2001–2011) in Korea. We compared the genomic contents of representative strains from the ST5-SCC*mec*II (SAV1022) and ST239-SCC*mec*III (SAV808) clones using draft genome sequencing and comparative genomic analysis. The results showed that the SAV1022 and SAV808 strains had the same gene related to vancomycin resistance, but the virulence and transport genes were different between strains. To investigate the virulence factor and cytotoxicity of MRSA from a domestic clinic, *S. aureus* ST1, ST5 and ST8 clones including MSSA and MRSA were tested. The *eno* and *fib* genes were differentially expressed between MSSA and MRSA, and the gene-expression of the ST1 type *cna*, and the ST8 type *clfA* and *fnbA* were noticeably higher than that of the others. It was confirmed that the MSSA cytotoxicity was higher than that of MRSA, and ST5 MRSA showed the lowest cytotoxicity. Each ST type in mice showed clearly different virulence between MSSA and MRSA, and the survival rate of mice inoculated with ST5 MRSA was higher than that of the other types of MRSA/MSSA. As a result, a correlation is suggested between the cell biological properties and virulence factor expression

To characterize the evolutionary pattern and genetic characteristics of MRSA, vancomycin resistant *Enterococcus faecium* (VREF), and *Acinetobacter baumannii* and to evaluate the mode of their dissemination in Korea, we collected 1,290 *S. aureus* strains over 12 years, 692 *E. faecium* over 11 years, and 1,077 *A. baumannii* strains from tertiary and nontertiary hospitals. Korean MRSAs have evolved into five dominant clones, and most Korean *E. faecium* have clonal complex 17 (CC17), including ST78, ST192, and ST203, which were distributed nationally. Korean *A. baumannii* has belonged to the globally-disseminated CC92 since 2009, among which ST191/2 were the most common STs.

The *vanA* cluster of *E. faecalis* was transferred to *E. faecalis* and *E. faecium* isolated from hospitals. However, that of *E. faecium* was transferred to *E. faecium* but not to *E. faecalis*. None of the VRE isolates transferred the *vanA* cluster to *S. aureus*. In a plasmid curing experiment using novobiocin and high temperature (42°C), plasmids of clinical VRE isolates were very stable. The vancomycin-resistant plasmid of clinical VRE was stable, but that of the transconjugant obtained from *E. faecalis* 5594 was unstable over 5 days. Spontaneous mutants obtained by exposure to various concentrations of rifampicin and fusidic acid did not grow on

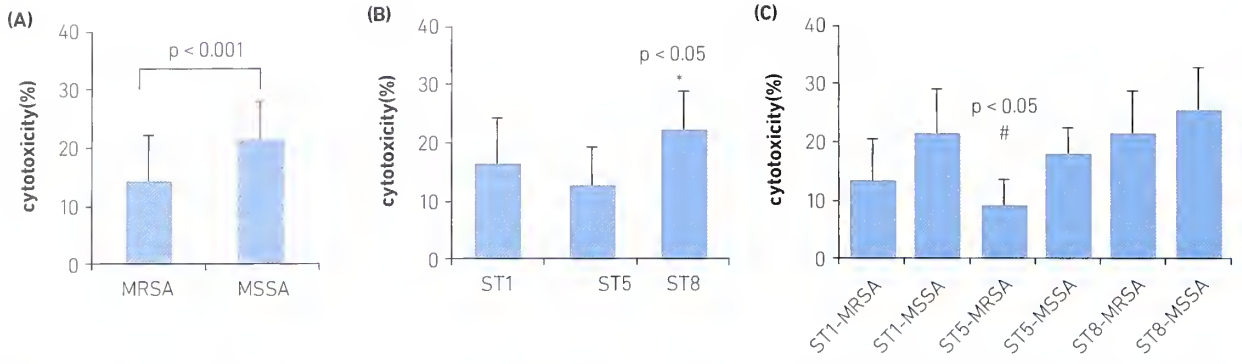


FIGURE 3. Cytotoxicity of Staphylococcus aureus to human lung epithelial cells. (A) methicillin-resistant Staphylococcus aureus (MRSA) vs. methicillin-sensitive S. aureus (MSSA) (B) Comparison to sequence type (ST), (C) MRSA vs. MSSA in ST type strains.

high concentrations of rifampicin (128 mg/L) or fusidic acid (96 mg/L). All *E. faecalis* isolates had high biofilm formation activity, but most of the *E. faecium* isolates had no or moderate activity.

In total, 312 isolates that had been reported as *A. baumannii* were selected for epidemiological characterization. However, only 76.3% were identified as *A. baumannii* and the others were *Acinetobacter spp.* using a molecular biological identification scheme. This result shows the significance of a molecular biological process in diagnosing a, *Acinetobacter* infection. *A. baumannii* isolates were characterized by two globally adopted MLST schemes proposed by the Bartual and Pasteur Institute. There were 18 STs including three new untypable STs, 24 STs, and four new untypable STs. They showed 22 profiles on a Bartual-Pasteur dual scheme analysis. They were grouped as Group I (73%) based on ST-BP 192-2 and Group II (3.5%) based on ST-BP 738-1 and seven singletons. A total of 181 carbapenem non-susceptible isolates were tested for carbapenemase genes; 168 isolates were positive against OXA-23 but they showed no OXA -24 or OXA-24 and no MBL type carbapenemase such as SIM, VIM, GIM. SPM, or IPM.

We analyzed 97 carbapenemase-producing Enterobacteriaceae isolated in 2011–2013. Two sequence types, ST258 and ST11, were identified in KPC-2 producing *K. pneumoniae*. The ST258 isolates showed high genetic similarity (>90%). The KPC-type enzyme genes were located on 45–145kb plasmids (IncFII, L/M and N). NDM-1 was detected in *K. pneumoniae* ST340, *E. coli* ST101, and *Enterobacter cloacae*. Most carried the IS*Aba125*-NDM-1-ble-trpF genetic structure. VIM-2 and GES-5 were identified in various species of Enterobacteriaceae. Most of the VIM-2 or GES-5 genes were located in the class I integron on the plasmids with the aacA4, aadA1, and orfII genes. The OXA-232 genes were located on the 6 Kb ColE type plasmid, and most were found in *K. pneumoniae* ST14.

Sixty *Neisseria gonorrhoeae* isolates were collected from clinical specimens of patients and prostitutes in 2013. None of the isolates tested were susceptible to penicillin G, and the rate of penicillinase-producing *N. gonorrhoeae* was 5% in 2013. All isolates were susceptible to ceftriaxone but one isolate was not susceptible to cefixme. The resistance rate of ciprofloxacin remained high (95%) and TRNG (18%) have been increasing recently. They showed different compared with previous data. Most *N. gonorrhoeae* were susceptible to azythromycin in 2013. None of the isolates were resistant to spectinomycin, and the minimum inhibitory concentration (MIC) of gentamicin was similar to that of a European study.

Sixteen strains were selected from clinical isolates in Korea using a carbapenem resistance test and molecular epidemiological methods. Specific genes detected in Korean isolates, and the gene structures surrounding beta-lactamase were compared among strains. An MLST simulation was conducted to compare similar strains using whole genome sequences, and antibiotic resistance genes were obtained using a database. As results, the specific beta-lactamase genes in Korean isolates were obtained, and insert sequences were detected after the resistance gene of selected isolates. These results can be used to analyze the mechanism of resistance and to conduct other studies such as a specific primer design for rapid detection of Korean isolates.

Antifungal susceptibilities and species distribution of 351 *Aspergillus* isolates from 11 university hospitals in Korea were investigated. They were separated in the order of *A. fumigatus* (42%), *A. niger* (23%), *A. flavus* (15%), and *A. terreus* (13%). The resistance of *Aspergillus* isolates against azole antifungal agents was < 3.0% and lower than those of foreign reports. Mutations in the cyp51A gene associated with azole resistance were not detected in any *A. fumigatus* isolate. A total of 55 clinical *Candida* isolates, of which the fluconazole MICs were above the ECVs, were genotyped by MLST and/or PFGE, and most of isolates showed unique genotypes except five *C. albicans* and two *C. glabrata*. Of the 20 *C. albicans* clinical isolates, mdr1 gene over-expression was found in 55%, and eight isolates showed amino acid substitutions in Erg11 related to azole resistance. All 12 *C. glabrata* isolates overexpressed the CgCDR1 and/or CgCDR2 genes. These findings show that clonal transmission of fluconazole-resistant *Candida* isolates is still very rare but possible. A total of 2,504 isolates from bacteremia cases and 1,673 isolates from urinary tract infection (UTI) cases were collected. The most frequent etiology for bacteremia was *E. coli* followed by *K. pneumoniae, S. aureus, P. aeruginosa* (respectively 35.0%, 15.3%, 14.6%, and 5.0%). *E. coli* (64.0%) was the most frequent etiology for urinary tract infections, followed by *K. pneumoniae* (9.3%), and *E. faecalis* (8.0%). Antimicrobial resistance of major bacteremia and UTI pathogens has increased significantly over the past 5 years. The increase in community acquired (CA)-MRSA was also remarkable. CTX-M-14 and 15 were the most frequent types of ESBL producing *E. coli* and *K. pneumoniae* and 35% of MRSA carried SCCmec type IV, and 61% of MRSA were ST72.

Antiviral agent development and an activity analysis were conducted against Enterovirus71 (EV71), which is the major antigen causing hand, foot, and mouth disease (HFMD) with neurological complications, particularly in children. No approved vaccine therapeutic agent against EV71is available. We used a high-throughput screening method with plant extract library to develop an antiviral agent against EV71. A *Hedera helix* extract showed antiviral activity against EV71. We confirmed that hederasaponin B was an effective antiviral compound after a high performance liquid chromatography experiment. We will continue to identify more antiviral agents against EV71 and reveal the hederasaponin B antiviral mechanism.

HIGH RISK PATHOGENS

Preclinical studies including stability, safety, and efficacy testing and a phase I human clinical trial were completed in 2009 to develop a human anthrax vaccine. A phase II clinical trial study plan was submitted to the Ministry of Food and Drug Safety in 2011, and step I of the human phase II clinical trial is in progress. We have also performed a study on poly ɣ-D-glutamic acid (PGA) capsule, which is a major *Bacillus anthracis* virulence factor. PGA has been reported to protect *B. anthracis* from immune surveillance by supporting the anti-phagocytic nature of the capsule. We produced monoclonal antibodies (mAbs) against PGA, and three-dimensional homology models with the cognitive PGA complex were analyzed (Figure 4). The anti-PGA monoclonal antibody showed a protective role in preventing progress of anthrax by inhibiting the enhanced cytotoxic activity of LT (lethal toxin) by PGA, as well as by eliminating the vegetative form of encapsulated *B. anthracis* through a specific interaction with the PGA capsule antigen.

Therapeutics for *Botulinum* neurotoxin (BoNT) intoxication consist of antibodies or antisera against BoNT. Efficient clearance of the BoNT-antibody immune complex determines the efficacy of the therapeutics. Here, we designed apoptotic cell-targeting therapeutic antibodies using an apoptosis cell targeting peptide-streptavidin conjugate with biotinylated anti-BoNT antibodies. The Apopep-1

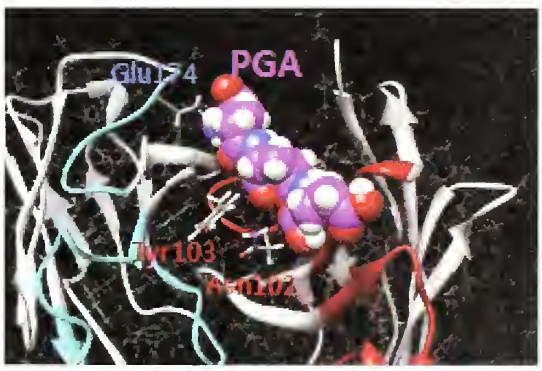


FIGURE 4. Binding site residues interacting with bound PGA. PGA binding pocket in the A-12 monoclonal antibody. The PGA antigen is surrounded by designated CDR residues. Pink color highlights the PGA backbones. Proposed key binding residues such as Asn102, Tyr103, and Glu174 in A-12 are shown in stick representation.

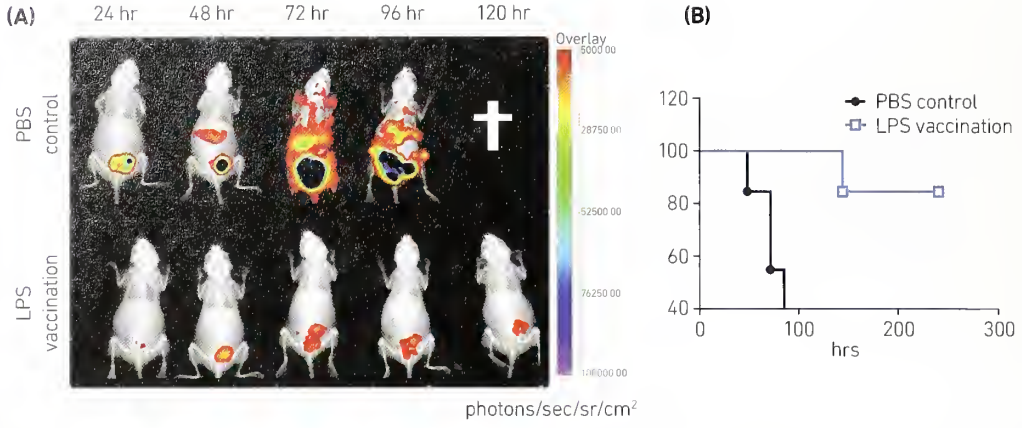


FIGURE 5. Real-time visualization of *F. tularensis* LVS in a live organism using bioluminescence signals. (a) In mice vaccinated with LPS, bacteria were detected only at the infection site and did not multiply until 120 hr after infection. (b) Survival rate of mice with or without LPS vaccination

peptide, which binds to the histone H1 protein on apoptotic cells, was chemically conjugated to maleimide-activated streptavidin to prepare a peptide-streptavidin conjugate. Fluorescein isothiocyanate (FITC) was incorporated for efficient tracing of the conjugate. The apoptotic cell-targeting feature was verified by tracing the conjugate on an apoptosis-induced murine macrophage cell line or primary cells by confocal microscopy. Specific targeting of the peptide conjugate was confirmed. We expect that these results might be relevant to realize efficient clearance of the BoNT-antibody immune complexes.

Yersinia pestis is a highly virulent facultative Gram-negative bacterium that causes bubonic and pneumonic plague. We investigated the effect of *Y. pestis* lipopolysacchride (LPS) on maturation and activation of dendritic cells (DCs). Mouse bone marrow-derived DCs were stimulated with LPS from *Y. pestis* grown at 27°C or 37°C. The DCs matured upon stimulation with 27°C or 37°C LPS by increasing the expression of maturation markers, although expression of markers by 27°C LPS was higher than that by 37°C LPS. Both 27°C and 37°C LPS stimulated toll-like receptor (TLR) 4 activation in Chinese hamster ovary cells expressing human TLR4 and CD14. Treatment of DCs with 27°C or 37°C LPS induced tumor necrosis factor (TNF)-α and interleukin (IL)-6 production. This induction of cytokine was completely abrogated in DCs from C3H/HeJ TLR4 mutant mice. The 37°C LPS induced phosphorylation of all three mitogen activated protein (MAP) kinases including ERK, JNK, and p38. In addition, 37°C LPS-induced TNF-α production decreased following treatment with inhibitors of ERK and JNK but not those of p38, whereas IL-6 production was attenuated by all three MAP kinases. The DNA binding activity of NF-κB p65 increased significantly in a dose-dependent fashion. These results suggest that LPS-induced cytokines production occurs by activating MAP kinases and NF-κB in mouse DCs.

We developed a bacteria tracing method that can analyze from the single cell level to the whole animal level, using an imaging system without a specific bacterial antibody. Using this method, we traced pathogenesis of *Francisella tularensis* in an organism and visualized bacterial replication in cells. Vaccination with LPS purified from *F. tularensis* live vaccine strain (LVS) greatly reduced bacterial replication of *F. tularensis* LVS. We expect that this novel analysis system will be useful for tracing high-risk pathogens and for *in vivo* analyses of infectious diseases, which have technical problems (Figure 5).

We developed a new nanotechnique-based immunoassay called the gold nanoparticle-based oligonucleotide-linked immunosorbent assay (GNP-OLISA) to enhance detection sensitivity. Current detection methods such as ELISA and real-time PCR for *F. tularensis* are not sufficiently sensitive to detect the infectious dose (about 10 colony-forming units) of *F. tularensis*. A novel detection method with highly advanced sensitivity was established, and the GNP-OLISA can be used as a method to detect the infectious dose of bio-terror agents.

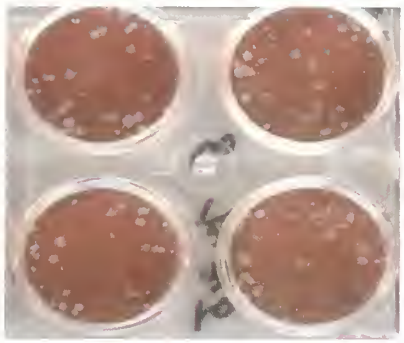


FIGURE 6. Plaque assay and skin test of new smallpox vaccine

VACCINE RESEARCH

We started to research for development vaccines against hand, foot and mouth disease (HFMD) caused by EV71. EV71 vaccine candidates were obtained and an vaccine candidate evaluation system was established. We developed EV71 vaccine candidates using a cell culture technique for clonal selection. Recombinant techniques were also used to develop the new vaccine candidates. An evaluation system for EV71 vaccine candidates was established for humoral and cellular immune responses in a mouse model.

We develpoped new small pox vaccine and viral vectors with other noble antigens. Detail data will be released after completion of patent application.

Human noroviruses (HuNV) are a major cause of food-borne gastroenteritis worldwide. There are no efficient cells or small animal models for HuNV. A few animal species have been described as models in previous studies. Among these animal models, the gnotobiotic (Gn) pig model shares significant similarities with human physiology, immunology, histo-blood group antigen (HBGA) phenotypes, and virus binding patterns, and provides an excellent animal model for studying HuNVs. We isolated the NV GII.4 Sydney 2012 variant and evaluated susceptibility of infection in specific pathogen-free (SPF) mini-pigs. Eight mini-pigs were inoculated with fecal filtrates of the NoV/GII_4/2012/Korea (2012 Sydney variants) strain, and the viral titer of the inoculums was about 3.0×10^9 genomic equivalents (GE) per pig. Diarrhea was not observed in inoculated pigs. About 75% (6/8) of the inoculated pigs shed virus in rectal swabs as detected by real-time RT-PCR from post-inoculation days (PID) 2–4 (average duration of 1.6 days, although one pig shed for 4 days). The virus titer peaked at 2.3×10^4– 5.7×10^5 GE/g from PID 1–4. Gastrointestinal contents (stomach, small intestine, and large

TABLE 2. Monitoring of virus shedding by real time reverse transcription-polymerase chain reaction

ID.of pigs	Strain of pigs	H/A type of pigs	Age (days 0OPI)	Dose/Head	fecal virus shedding(copies/g)					Mean onset of vital shedding (DPI)	Mean Duration of fecal Virus shedding (day)
				Copies	0DPI	1DPI	2DPI	3DPI	4DPI	5DPI	
1	Hanford	A	12-14	2.62E+09	ND	ND	1.17E+05	2.57E+05	4.34E+05	ND	2	3
2	Hanford	A	12-14	2.31E+09	ND	ND	ND	ND	ND	ND	-	-
3	Hanford	A	12-14	5.73E+08	ND	ND	ND	2.29E+04	7.40E+04	ND	3	2
4	Hanford	A	12-14	2.86E+08	ND	2.45E+05	2.28E+05	1.39E+05	1.58E+05	ND	1	4
5	White Yucatan	A	18-28	3.62E+09	ND	ND	5.67E+05	-	-	-	2	-
6	White Yucatan	A	18-28	3.62E+09	ND	ND	ND	4.99E+05	ND	ND	3	1
7	White Yucatan	A	18-28	2.59E+09	ND	ND	ND	ND	ND	ND	-	-
8	White Yucatan	A	18-28	2.59E+09	ND	ND	ND	5.11E+04	ND	ND	3	1

* ND, not detected

intestine) and intestinal tissues (duodenum to colon) were confirmed by real-time RT-PCR. We detected HuNV RNA in all gastrointestinal contents, and the virus titer was 2.0×10^6–8.7×10^6 GE/g. Among the intestinal tissues, NV was only detected in the ileum (2.6×10^5 GE/g). These results suggest the SPF mini-pig as an animal model for HuNV infection.

Laboratory Surveillance

LABORATORY SURVEILLANCE FOR ENTERIC PATHOGENS

Acute Gastroenteritis Surveillance (EnterNet-Korea)

EnterNet- Korea, a web-based reporting system, was established in 2007 to improve laboratory activities and enhance reporting proficiency. This system and comprised 17 province environmental and health institutes and 105 participating hospitals. Target pathogens include 10 genera of bacteria and five types of viruses.

From 2009, this system was re-constructed for precise reporting by several laboratory data. In 2013, 20,984 stool samples were collected resulting in 3,668 pathogen isolations (17.5%). Among the isolates pathogens, *S. aureus* accounted for 972 cases, pathogenic *E. coli* for 954, *Salmonella* for 523, and *Campylobacter jejuni* for 158.

Among the viral pathogens detected in 2013, group norovirus was the most common, at 56.1%, whereas rotavirus, enteric adenovirus, astrovirus, and sapovirus were found at frequencies of 28.6%, 10.2%, 4.1%, and 1.1 %, respectively.

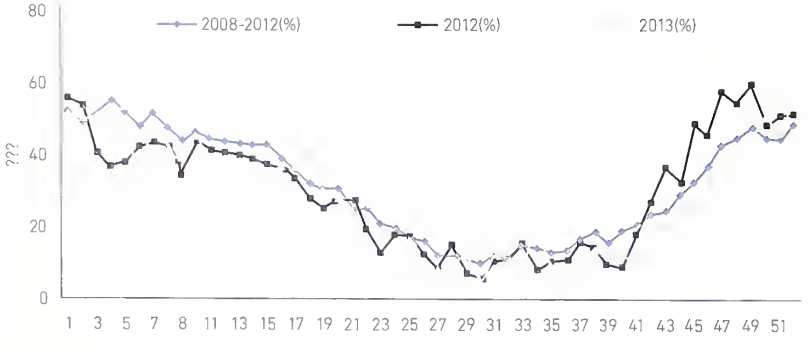


FIGURE 7. Seasonal incidence of diarrheal viruses causing acute gastroenteritis

The predominant viral genotypes identified in Korea during 2013 were GII-4 and GII-3 among norovirus infections, and G1P[8], G2P[4], G3[8], and G4P[6] among rotavirus infections. The majority of adenovirus and astrovirus serotypes were determined to be serotypes 41 and 1, respectively.

In future plans, target pathogens via the changed legal pathogen list will be increased and the surveillance of acute diarrheal disease resulting from imported pathogens will be enforced. In addition, research on risk evaluation and the response to risk factors will be improved.

PulseNet (PN) Korea

PN is a bacterial pathogen-tracing network for infectious disease surveillance based on molecular subtyping. In 2005, the KNIH inaugurated PN Korea. PN Korea consists of the KNIH, as a coordinating center, and participating laboratories including the Korea Food and Drug Administration, the National Veterinary Research and Quarantine Station, and the Institute of Health and Environment in each provincial government. Construction of the PN Korea network was completed in 2007, and all participating laboratories possess the PFGE system and BioNumerics software that are used for the web-based PFGE data analysis system and database construction.

A total of 12,569 PFGE results for 15 pathogens have been entered into the PN Korea database to date, including a case of early prevention of the 2011*Salmonella Enteritidis* epidemic. Various other important molecular epidemiological analyses of food-borne infectious disease cases were conducted and distributed to the relevant public health authorities in a PN report. PN Korea also developed next-generation molecular subtyping methods, called multi-locus variable tandem assay (MLVA) for *Shigella sonnei*, *S. flexneri*, *S.* Enteritidis, *S.* Typhimurium, and *E. coli* O157:H7. This MLVA

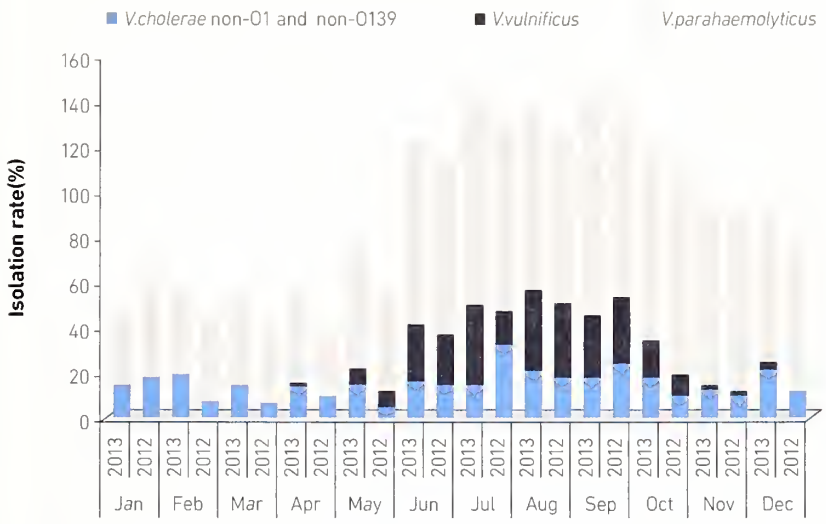


FIGURE 8. Detection of *Vibrio cholerae*, *V. vulnificus* and *V. parahaemolyticus* by month during 2013

method should complement PFGE and strengthen cooperation with the international PN network.

We plan to renovate and distribute various guidelines (PFGE and MLST manuals) for PN Korea and develop a MLVA method for *S.* Typhimurium. Additionally, we plan to develop a genetic marker related to the prevalence of shigellosis in *S. sonnei*.

K-VibrioNet

K-VibrioNet was initiated in 2005 and consists of the KNIH, 11 national harbor area quarantine stations, and four Institutes of Health and the Environment. Using this network, we have attempted to elucidate the distribution of pathogenic *Vibrio* spp. in Korean coastal areas and to identify the relationship between this distribution and the incidence of vibriosis. We confirmed the distribution patterns of *Vibrio cholerae*, *V. vulnificus*, and *V. parahaemolyticus* according to water temperature and region. The operational method of this network

TABLE 3. PulseNet Korea Database from 2005 to 2013

Pathogens name	~2005	2006	2007	2008	2009	2010	2011	2012	2013	Cumulative
S.typhi	442	245	82	120	29	28	25	15	10	996
S.Enteritidis	4	56	247	424	443	208	286	132	169	1,978
Other *Satmonella*		93	44	65	25	82	106	192	154	761
S. sonnel	735	113	46	94	21	14	22	48	151	1,244
S. flexnerl	102	19	6	155	123	56	6	6	6	471
S. dysenteriae	9									9
EHEC		40	25	107	97	30		10		309
EHEC				8	31		52	55	76	222
EHEC	12			30	63	19		33	170	327
EHEC	50		37	47	12	61	77	109	287	680
E.coli	108	79				29	12	5		233
V. cholerae	55	38	99	144	68	68	318		11	801
V. parahoemolyticus			338	330	328	78	421	51		1,546
V. vutnificus		152	100	236	58		50			594
C. jejuni						42	588	92	75	797
S. aureus								219	66	219
Other			197	296	204	269			22	988
Total	**1517**	**844**	**1221**	**2056**	**1500**	**984**	**1963**	**1,253**	**1,269**	**12,531**

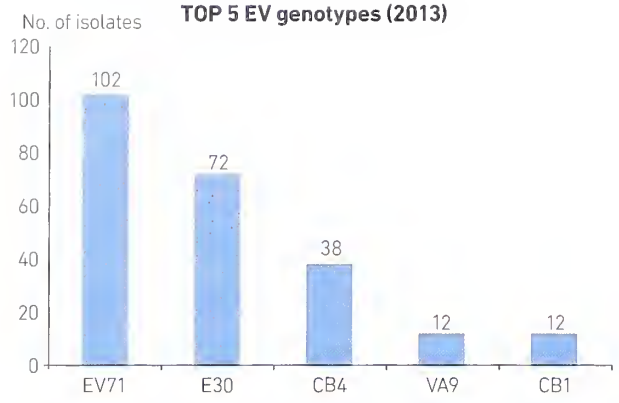


FIGURE 9. Genotypic distribution of Enteroviruses in Korea, 2013

has been improved continuously. Furthermore, we developed a mathematical model to estimate potential *Vibrio* occurrence. This mathematical model will improve VibrioNet, making it an appropriate system for use in preparation for climate change events, including global warming.

Laboratory Surveillance of Enteroviruses

The Division of Vaccine Research manages a national enterovirus surveillance system. The enterovirus network processes stool and cerebrospinal fluid (CSF) as well as respiratory, skin, blood, and pericardial fluid specimens. Seven Regional Institutes of Health and Environment (RIHEs) detected the enterovirus by real-time RT-PCR and VP1 sequencing. Physicians submitted specimens collected from patients suspected of enterovirus infection, based on symptoms including flu-like illness, meningitis, encephalitis, HFMD, herpangina, and gastroenteritis, along with information pertaining to patient age, date of specimen collection, clinical symptoms, and suspected diagnosis. Analysis of the specimens, including typing of relevant enterovirus and other characterizations, was carried out at the National Polio Laboratory (NPL) of the KCDC. A total of 2,396 specimens with different syndromes were collected by the national enterovirus surveillance system in 2013. Of the 2,396 specimens, 911 (38.0%) tested positive for human enterovirus (EV), and EV71, E30, CB4, CA9, and CB1 ranked as the top five genotypes identified.

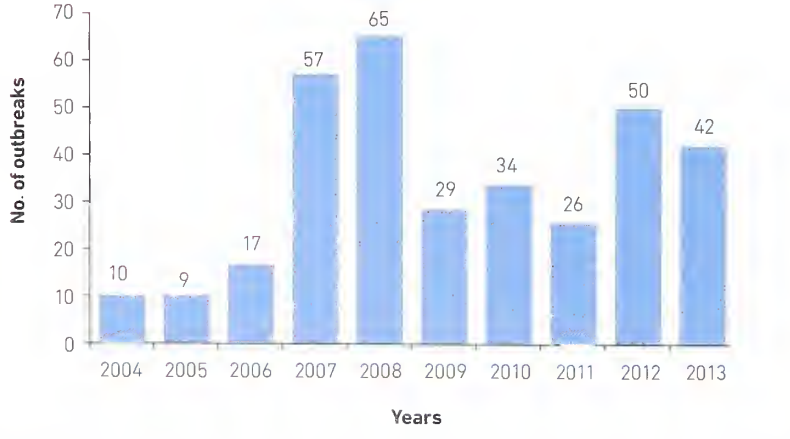


FIGURE 10. Yearly incidence of norovirus -related outbreaks in Korea, 2004–2013

National Surveillance of Norovirus-Related Outbreaks

We actively monitored gastroenteritis outbreaks using a web-based reporting system for norovirus molecular epidemiology named K-CaliciNet to strengthen regional laboratory activities by supporting the reporting and tracing of related outbreaks. Epidemic norovirus infections have increased drastically since 2006. Of the 261 outbreak cases, 42 (16%) were determined to be norovirus-related epidemics after identifying the causative agents, revealing that norovirus was the predominant agent causing food-borne outbreaks in Korea in 2013.

We standardized the norovirus detection method of the pan-governmental section to perform effective nationwide viral gastroenteritis surveillance. Thus, real-time RT-PCR was standardized for the first time to decrease variability in molecular methods.

The K-CaliciNet web-based reporting system has been used since 2007 for sequence analysis to determine the genetic identity of noroviruses causing food-borne disease outbreaks. Sequences of noroviruses that cause diseases throughout Korea were entered into K-CaliciNet to rapidly assess the relationships among circulating strains. To date, 15,538 norovirus sequences, including 35 subtypes, have been collected in the K-CaliciNet database.

LABORATORY SURVEILLANCE OF RESPIRATORY PATHOGENS

Korea Influenza and Respiratory Viruses Surveillance System (KINRESS)

The Division of Influenza Virus and the Division of Respiratory Viruses implemented an active

TABLE 4. Norovirus sequence database, 2008–2013

case	No. of norovirus sequence database						
	2008	2009	2010	2011	2012	2013	Sub-total
Sporadic case	1,614	3,599	2,670	1,974	2,078	1,129	13,064
Outbreak case	182	587	204	350	546	605	2,474
Total	1,796	4,186	2,874	2,324	2,624	1,734	15,538

nationwide surveillance network in May 2009, called the Korea Influenza and Respiratory Viruses Surveillance System (KINRESS), to monitor acute respiratory infections (ARIs). In total, 108 participating hospitals in the 16 provinces collected throat or nasal swabs from enrolled outpatients with ARIs including influenza-like illness (ILI) for 1 year. These samples were analyzed by real-time RT-PCR for eight respiratory viruses (15 subtype viruses) at the 17 provincial research institutes of *the* health and environment (Figure 11).

A total of 15,050 patients (ages range, < 1–92 years) were enrolled in 2013. More than 75.0% of the patients had fever and 58.1% had cough. Of the eight tested respiratory viruses, human rhinovirus (HRV) and influenza virus had the highest detection rates of 2,411 (16.0%) and 1,773 cases (11.8%), respectively. HRV was detected year-round, with a peak occurring during late summer and fall. The parainfluenza virus and human bocavirus were prevalent from April to August. The number of respiratory syncytial virus (RSV) infections began to increase during late fall and peaked between October and December (Figure 12). Among 1,773 influenza viruses, A(H3N2) viruses (1,273, 71.8%) were predominantly circulating during 2012. A(H1N1)pdm09 (322, 18.2%) and B (178, 10.0%) were detected to a lesser extent than that of A(H3N2) (Figure 28). The antigenic characteristics of the majority of influenza viruses were closely

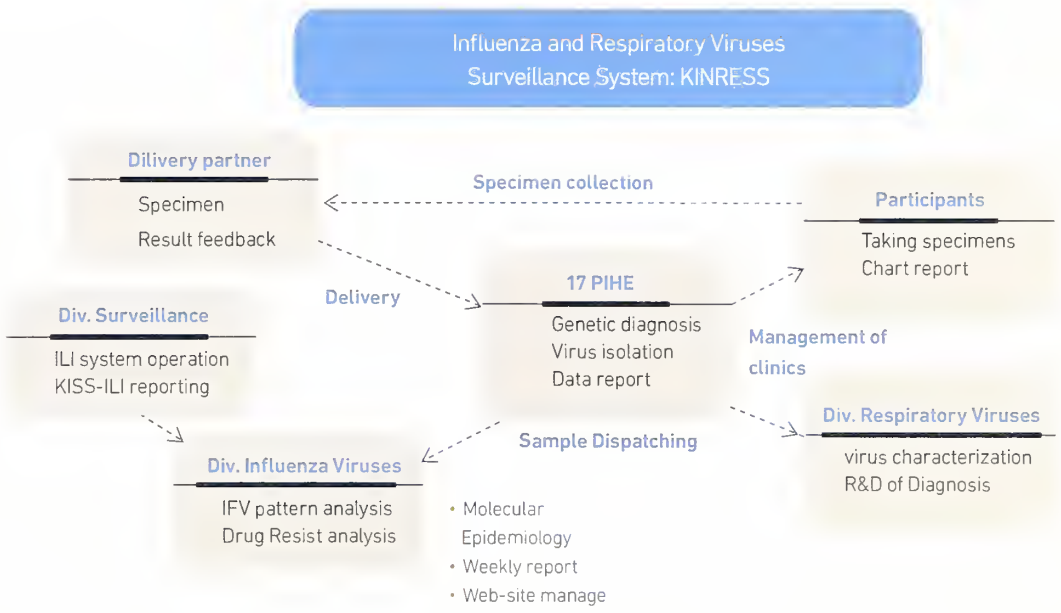


FIGURE 11. Schematic flow chart for KINRESS

TABLE 5. The number of influenza virus isolates and antiviral drug resistance during 2013

Virus isolates		Antiviral resistance	
		NA tested[Resistant]	M2 tested[Resistant]
A(H1N1)pdm09	322	176 (0)	176 (0)
A(H3N2)	1,273	440 (0)	440 (0)
B	178	110 (0)	-
Total	1,773	726 (0)	616 (0)

related to the vaccine strains. No neuraminidase (NA) inhibitor- resistant virus was found. However, all influenza type A viruses were resistant to an M2 inhibitor (Table 2). A KINRESS workshop was held on November 5–6, 2013, at Gyeongju to share related information and to improve the laboratory surveillance system. Sixty-one attended and nine topics were presented including national and regional laboratory surveillance data.

The clinical characteristics of respiratory virus infections will be investigated in the near future by analyzing the KINRESS data. The laboratory diagnostic system for respiratory viruses will be upgraded to a real-time PCR/RT-PCR system to improve virus detection sensitivity and specificity. A genetic analysis system will be used in the 17 provincial laboratories for the rapid detection of influenza virus antigenic variance and antiviral resistance. These diagnostic and surveillance efforts should be continued for upcoming novel influenza viruses.

Acute respiratory infection (ARI) Net

The ARI Net is a surveillance network for pathogens in ARIs. This surveillance network is divided into 2 sub-networks, upper respiratory tract infections (URIs) and lower respiratory infections (LRIs). In 2013, pathogen distribution in community acquired pneumonia (CAP) and acute bronchitis were detected through LRI surveillance system. Pathogens in pharyngitis infection were also detected in URI surveillance system. Specimens were collected from suspected patients and the etiological agents were confirmed by laboratory diagnostic tests. In addition, the antibiotic resistance pattern of the isolated pathogens was confirmed.

Community Acquired Pneumonia (CAP) Network

A total of 1,729 specimens were collected from enrolled patients during the surveillance period (2009–2013). The positive rates were 24.3% for bacterial pathogens, 21.5% for atypical bacterial pathogens, and 36.3% for viral pathogens. Sputum specimens were collected from 217 patients at 22 participating hospitals in 2013. The pathogens tested included 10 bacterial pathogens, three atypical pathogens, and 10 viral pathogens. In the group of bacterial pathogens, *S. pneumoniae* (6.9%), *H. influenzae* (2.3%), and *K. pneumoniae* (3.7%) showed higher positive rates. In the viral pathogens, influenza virus (10.1%) and human rhinovirus (12.0%) showed higher frequencies. In particular, the positive rate of atypical pathogens was greatly reduced to 2.8% from 14.7% in 2012. *S. pneumoniae* showed resistance to azithromycin, erythromycin, tetracycline and cefuroxime. Higher resistance to ampicillin and penicillin was observed by *S. aureus*, and *H. influenzae* showed resistance only to ampicillin and trimethoprim/sulfamethoxaszole.

Acute Bronchitis Network

In total, 170 patients were enrolled in the acute bronchitis surveillance network. Similar to the CAP network, 10 bacterial pathogens, three atypical pathogens, and 10 viral pathogens were identified by laboratory tests. The positive rates in each pathogen category were 31.2% for bacterial pathogens, 1.2% for atypical pathogens, and 29.4% for viral pathogen. In particular, the positive rate for atypical pathogens was greatly reduced compared to the result in 2012. Among bacterial pathogens, *H. influenza* (12.4%) and *S.*

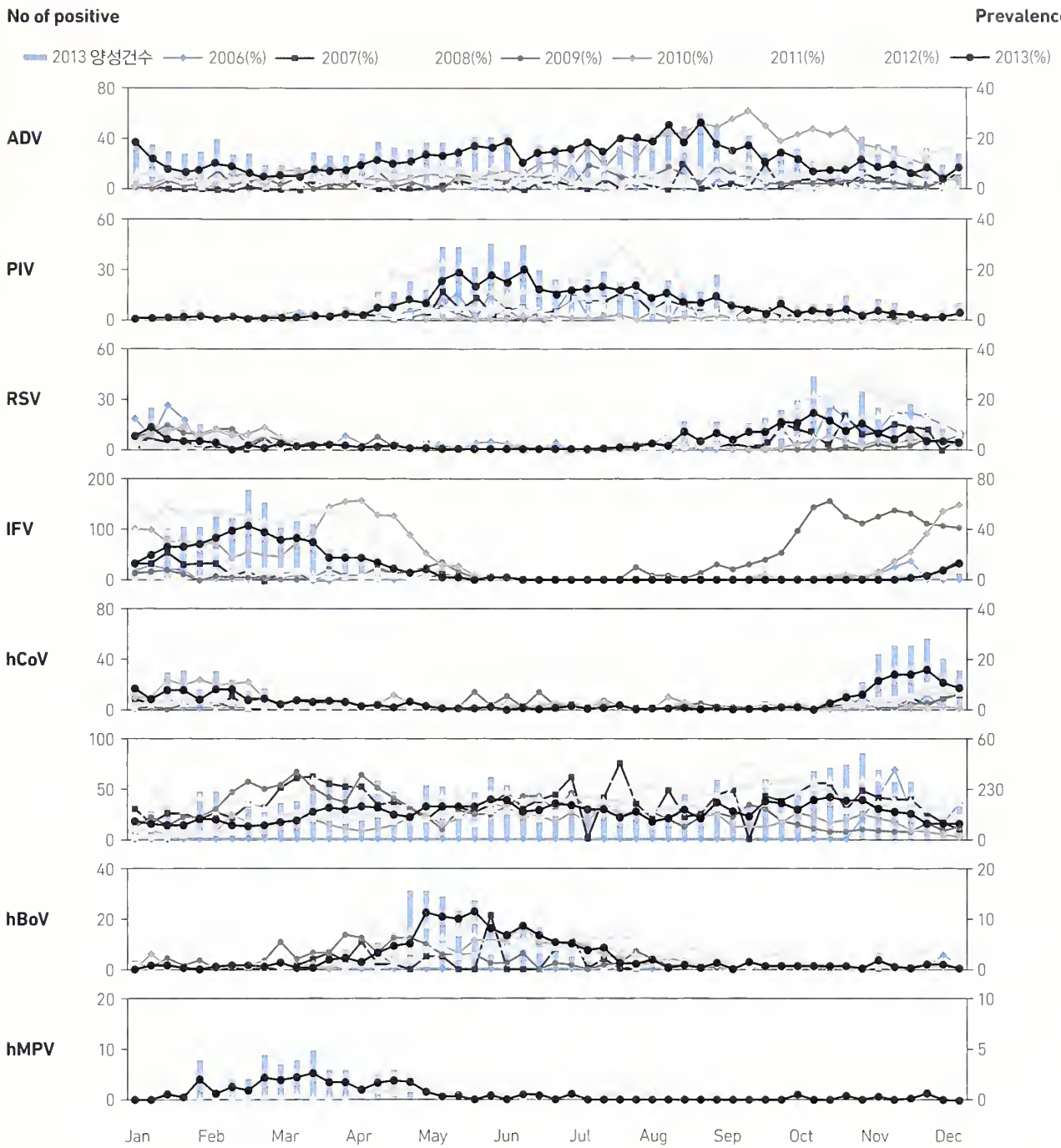


FIGURE 12. Seasonal incidence of respiratory viruses causing acute respiratory infections based on figures released weekly through the Korea Influenza and Respiratory Viruses Surveillance System Report published by the KNIH, KCDC

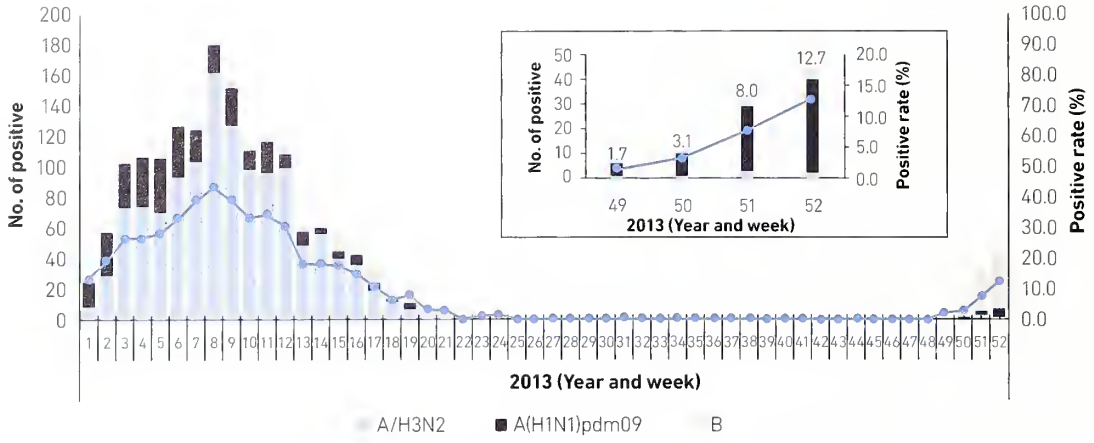


FIGURE 13. Detection of influenza viruses, by week, during 2013

pneumoniae (5.9%) showed higher frequencies than those of other bacterial pathogens (Table 6). In the case of viral pathogens, rhinovirus (13.5%) showed the highest frequencies, similar to the result in 2012. In addition, parainfluenza virus (6.5%) and RSV (4.7%) showed relatively higher frequencies.

TB COHORT OF HIGH-RISK POPULATION

TB is the most serious infectious disease in the Republic of Korea. The tuberculin skin test (TST) has been widely used to diagnose a latent TB infection (LTBI). Interferon gamma release assays (IGRA) using a *M. tuberculosis* specific antigen have been increasingly used and investigated worldwide. Preventing development of the disease among infected persons and blocking new infections are important to reduce the occurrence of TB. It is important to properly estimate the risk of incidence within a high-risk population to expand preventive treatment, which may complement the current national guidelines for treating LTBI. Therefore, it is essential to conduct a cohort study of high-risk populations infected with *M. tuberculosis* to satisfy the following objectives: 1) To identify the incidence of TB among recently exposed close TB contacts in Korea; 2) to evaluate risk factors for TB and determine relative risk after adjusting for major confounders; 3) to compare the IGRA with TST in recently-exposed contacts of active pulmonary TB cases with respect to their development of TB; 4) to collect data derived from blood samples and isolates from participants for research purposes.

A total of 3,000 close contacts of patients with smear-positive pulmonary TB, aged between 7 and 40 years, were recruited from schools and healthcare centers over 5 years. The enrolled subjects were asked to indicate whether they had previously received a BCG vaccination and to report

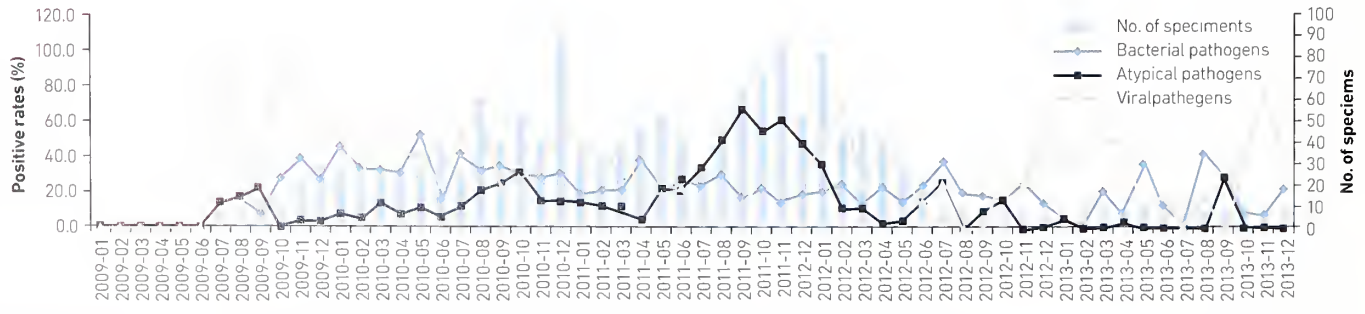


FIGURE 14. Analysis of monthly positive rates according to their laboratory-confirmed pathogens

TABLE 6. The positive distribution of pathogens in patients with acute bronchitis in Korea, 2013

Bacteria	No.(%) of Positive cases	Vial pathogens	No.(%) of Positive cases
S. pneumoniae	10(5.9)	Adenovirus	1(0.6)
H. influenzae	21(12.4)	Parainfluenza virus	11(6.5)
M. catarrhalis	2(1.2)	Respiratory Syncitial virus	8(4.7)
S. aureus	5(2.9)	Influenza virus	2(1.2)
K. pneumoniae	11(6.5)	Human Coronavirus	3(1.8)
P. aeruginosa	4(2.4)	Human Rhinovirus	23(13.5)
Group A *Streptococcus*	0(0.0)	Human Bocavirus	0(0.0)
Group B *Streptococcus*	0(0.0)	Human Enterovirus	1(0.6)
A. baumanii	0(0.0)	Human Metapneumovirus	1(0.6)
Total	53(31.2)	Total	50(29.4)

Atypical pathogens	No.(%) of Positive cases	Atypical pathogens	No.(%) of Positive cases
C. pneumoniae	0(0.0)	*B. pertussis*	5(0.2)
M. pneumoniae	2(1.2)		
Legionella spp.	0(0.0)		
Total	2(1.2)	Total	5(0.2)

details of TB history, or any family history of TB. In addition, a chest X-ray, TST, and IGRA (Quantiferon-Gold in a tube) were performed. Follow-ups were conducted 3–6 months and 1 year after the first examination (school setting), and within 1 year from the second follow-up, and then traced for up to 10 years using a surveillance system. Contact subjects developing culture-confirmed TB were analyzed by IS6110 restriction fragment length polymorphism fingerprinting.

A total of 3,088 contacts were recruited and analyzed from April 2008 to October 2012. The proportions of positive reactions were 23.0% and 11.5% for TST (≥10 mm) and IGRA, respectively. The results showed that only 36.9% of TST positive responders were IGRA positive responders (kappa index; 0.40, 95% confidence interval [CI], 0.36–0.44). IGRA positivity was significantly lower in subjects with a BCG scar than in those without a BCG scar. Age was the only significant factor affecting tuberculin positivity (≥10 mm). However, age, sex, and the presence of a BCG scar affected IGRA positivity.

We detected 27 patients with TB (0.87%) by tracing recruited contacts. When comparing IGRA with TST with respect to the development of TB, 1.97% of the TST-positive contacts and 3.66% of IGRA-positive contacts developed the disease, indicating that IGRA has higher predictive value for the development of TB than that of the TST. The data analysis showed that TST and IGRA positive responders had 5.89 (95% CI, 2.63–13.15) times more risk for developing TB, demonstrating that this group should be subjected to preventive

chemotherapy as described in the current national guidelines. However, 11 contacts who developed active TB were initially negative on both tests, but eight converted to positive on follow-up tests. Therefore, repeating tests for negative responders within a window period should be considered in the South Korean NTP guidelines. Sex, IGRA result, and family history of TB were related to the development of TB. BCG vaccination and TST results did not correlate with development of the disease.

LABORATORY SURVEILLANCE FOR ANTIMICROBIAL RESISTANCE

Korea Antimicrobial Resistance Monitoring System (KARMS)

We developed an antibiotic resistance data collection system for tertiary and non-tertiary care hospitals. The antimicrobial resistance rate of major infectious disease organisms has been analyzed for 6 years (2007–2012). The resistance rate of *S. aureus* to methicillin has remained high for the past 6 years. Vancomycin-resistant *E. faecium* was more prevalent than vancomycin-resistant *E. faecalis*. According to the tertiary care hospital data, the carbapenem resistance rate of *A. baumannii* increased from 67.1% (2011) to 69.5% (2012) and that of cefotaxime-resistant *E. coli* was 29.0% (2012), while ceftazidime-resistant *K. pneumoniae* was 40.0%(2012). The antimicrobial resistance rate of major antimicrobial resistant organisms was analyzed for 6 years (2007–2012), and this information was published in the KARMS 2012 annual report of antimicrobial resistance in Korea.

The prevalence and resistance mechanisms of medically important Gram-positive and -negative bacteria (MRSA, VRE, and multi-resistant Gram-negative bacilli) were studied in clinical isolates from 35 university and general hospitals in Korea in 2013. Carbapenem resistant *P. aeruginosa* was mainly due to the production of IMP-6 carbapenemase. VanA gene was found in 32.1% of *E. facieum*. The rate of MRSA was 67% and that of vancomycin-resistant *E. faecium* was 31%. The rate of cefotaxime-resistant *E. coli* was 28%, and that of ceftazidim and carbapenem resistant *K. pneumoniae* were 39% and 1.0%, respectively. The rates of imipenem resistant *P. aeruginosa* and *A. baumannii* were 30% and 75% respectively.

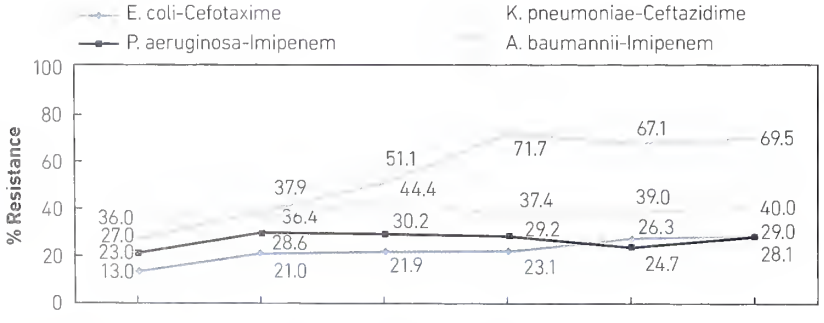


FIGURE 15. Resistance trends of Gram-negative bacilli from general hospitals

Laboratory Surveillance Systems for VRSA and CRE

We investigated the prevalence of vancomycin-intermediate *S. aureus* (VISA) among MRSA strains isolated from clinical samples at tertiary and general hospitals participating in a nationwide surveillance program for VISA and VRSA in Korea during a 12 week period in 2013. Of the 28,292 MRSA isolates, 20,242 were screened, 43 grew on brain heart infusion agar supplemented with 4 μg/ml vancomycin, and 10 VISA isolates were confirmed by the broth microdilution method, E-test, and agar dilution method. Seventeen VISA isolates were found from the surveillance program and confirmation requests in 2013. No VRSA isolates have been detected in Korea until now. Molecular epidemiology of 17 VISA isolates revealed that the ST5-MRSA-SCC*mec* II strain was the most prevalent (94.1%).

We performed a confirmation test for carbapenemase producing Enterobacteriaceae (CPE) isolates from tertiary care hospitals, non-tertiary care hospitals, and geriatric hospitals. A total of 116 CPE isolates were found in 2013, compared to 44 in 2012. Among the isolates, 53 OXA-232 carrying Enterobacteriaceae were identified, including 32 NDM-1, 15 VIM-type, 9 KPC type, 6 IMP-typem and 1 GES-5 carrying isolate. *K. pneumoniae* was the predominant CPE (68.1%, 79 isolates). OXA-232 (only one amino acid change from OXA-181) producing *K. pneumoniae* was isolated for the first time in a Korean hospital. The isolate was recovered from a patient transferred from India to Korea. The OXA-232 gene was located on the ~6Kb ColE-type plasmid. Plasmids of some *K. pneumoniae* isolates were conjugative to *E. coli* J53.

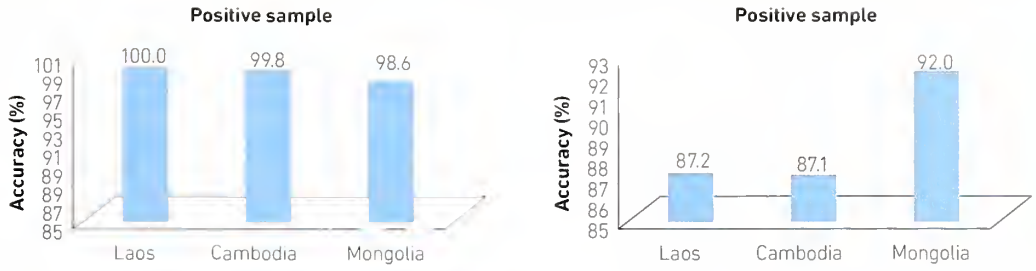


FIGURE 16. Accuracy of the detection assays from three national reference laboratories in the World Pacific region, 2013

WHO REGIONAL OR NATIONAL REFERENCE LABORATORY

WHO RoV Regional Reference Laboratory in Western Pacific Region

The Division of Vaccine Research in KCDC was designated as a regional reference laboratory (RRL) in the Western Pacific Regional Office (WPRO) for a rotavirus global network in April 2010. A pre-existing nationwide surveillance system for monitoring rotaviral gastroenteritis had generated laboratory-based surveillance data to show the prevalence of rotavirus infection and the genotype distribution. We applied this infrastructure for diagnosing and molecular typing of rotaviruses in Korea to function as an RRL. Three Asian Pacific countries (Mongolia, Laos, and Cambodia) were selected by the WPRO to operate a surveillance network in the WPR region. The RRL should act as the reference laboratory for diagnostic testing in the rotavirus global network. Therefore, we evaluated the proficiency of screening tests for rotavirus infections performed previously by each national lab, and genotyped rotaviruses circulating in the three countries to identify the predominant rotavirus strains in the WPR. Under contract between the WHO and the KCDC (the chief of the Division of Vaccine Research), we received 1,174 stool specimens, including 832 rotavirus-positive and 17 borderline samples, and 325 negative samples from the three national reference laboratories (NRLs) for further genetic characterization and evaluation of test accuracy for detecting rotavirus antigen. We confirmed a 99.34% consistency (827 of 832) among rotavirus-positive specimens and 89.2% consistency (290 of 325) among negative samples. Of the 878 rotavirus ELISA-positive samples, four types of human strains, including P[8]G1, P[4]G2, P[8]G3, and P[8]G9, were common and represented 81.32% (n=714) of rotavirus infections. P[8]G1, which is the most common human strain, was the most prevalent strain in Cambodia, and P[8]G3 was detected at the highest frequency in Laos. The P[8]G3, P[6]G9 strains circulated at a high frequency in Mongolia during the 2012–2013 season. Molecular epidemiological information obtained from our 2013 activities as an RRL provides a review of the current endemics for infectious gastroenteritis, such as those caused by rotavirus. Moreover, useful information has been provided to develop disease control strategies for rotavirus infection in the WPR.

WHO/WPRO Regional Reference Laboratory for invasive bacterial vaccine preventable diseases (IB-VPD)

The WHO has established a global surveillance for IB-VPD to provide quality data to assist countries with planning of public health programs in making decisions on introducing vaccines into their national immunization programs, to help demonstrate the need for vaccination, and provide up-to-date baseline data to assess the impact of vaccines on disease trends following introduction of a vaccine. To support surveillance requirements for diagnosis and confirmatory testing, the Division of Bacterial Respiratory Infections, designated as RRL in the WPRO, performed detection and serotyping/serogrouping of IB-VPD pathogens (*S. pneumoniae, Haemophilus influenzae, and Neisseria meningitidis*) in specimens collected from ongoing pneumonia, sepsis, and meningitis surveillance in Mongolia and Cambodia.

We performed real-time PCR detection of IB-VPD pathogens from CSF specimens collected in Mongolia and Cambodia during 2013. Among 42

CSF specimens from Mongolia, 38 were positive for three pathogens (seven positive for *S. pneumoniae* and five positive for *N. meningitidis*). The *S. pneumoniae* serotypes were types 2, 5, 6A/B, 19F, and 23F, and the *N. meningitidis* serogroups were groups B and W135. These results were provided to the referring laboratory and the WHO Regional Office in a timely manner. We are performing confirmatory testing of blood broth samples using real-time PCR and conventional multiplex PCR. We participated in the annual quality assurance program for IB-VPD RRL in June 2013.

To maximize identification and serotyping/serogrouping of IB-VPD pathogens, we participated in the laboratory technical working group meeting for the IB-VPD surveillance network in Bangkok in November 2013 and undertook a strategic review of the algorithm prioritizing testing of CSF specimens sent by national laboratories to RRL.

National Influenza Center (NIC)

The WHO Global Influenza Surveillance and Response System (GISRS) has a history of over 50 years. WHO GISRS monitors the evolution of influenza viruses and provides recommendations for laboratory diagnostics, vaccines, antiviral susceptibility and risk assessment.

National Influenza Centers (NICs) were designated by the Ministry of Health and recognized by the WHO for the Global Influenza Program. The Division of Influenza Virus of the Korean National Institute of Health was designated as the NIC in 1971. Since then, the Korean NIC has served as the key contact point and has maintained active communication as a member of the WHO GISRS through timely submission of viruses, providing immediate information on isolation of unusual viruses or disease outbreaks, and preparing weekly reports on influenza activity during the influenza season. The Korea NIC has also been participating in the WHO External Quality Assessment Program to improve diagnostic quality for detecting influenza A virus using molecular diagnostics.

WHO National Measles Laboratory

As the National Measles Laboratory (NML) of Korea, denominated by the WHO, the Division of Respiratory Viruses in the Korea National Institute of Health (KNIH) performed a serological diagnostic quality assurance program targeting measles laboratory networks in Korea. The major accomplishments of the program include improvements in the accuracy and timeliness of measles diagnoses by public and private measles laboratories in Korea. The KNIH participated in molecular and serological proficiency testing programs governed by WHO/WPRO to ensure high technical quality and consistent laboratory performance as the NML and obtained 100% of the points in 2013 proficiency testing programs. Seventeen province-level laboratories were assessed annually for serological and virological laboratory performance, including detection of measles/rubella specific IgM in serum, detection of viral RNA in clinical samples, and isolation of viruses in cell culture by the Korean National Measles Laboratory, KNIH. Five major private clinical laboratories also voluntarily participated in the serological performance quality control program. Future plans include strengthening the NML network activities. All 22 of the measles laboratories in the network will be improved and an annual quality assurance program will be conducted. The measles surveillance system, including the active laboratory surveillance system, will be strengthened to rapidly detect the measles virus and specific measles antibody. The characteristics of measles isolates in Korea will be investigated by full sequence analysis and virological characterization.

WHO Poliovirus National Reference Laboratory

The Division of Vaccine Research in the KCDC was designated as the NRL of the Republic of Korea for polio-free certification in the Republic of Korea. The Division of Vaccine Research has managed the acute flaccid paralysis (AFP) surveillance system to monitor import of wild polioviruses from endemic regions since 1998. Physicians submit two stool specimens collected from patients with AFP at least 24–48 hr apart and within 14 days of the onset of paralysis along with patient age, date of specimen collection, clinical symptoms, and suspected diagnosis. Virological testing of stool samples from patients with AFP is standardized in the WHO laboratory network.

Eighty-four AFP cases were reported through the AFP surveillance system, but no wild polioviruses were reported, and 72 cases were positive for non-polio enterovirus (NPEV) in 2013. EV71 was the most frequently observed (51 of 76 cases, 67.1%), which is associated with Guillain-Barré Syndrome, HFMD, meningoencephalitis, meningitis, and transversemyelitis. The laboratory accreditation system for maintaining a polio-free status in Korea is approved by a proficiency test and an annual visit

from the WHO/WPR. The National Polio Laboratory in Korea was fully accredited by the WHO/WPRO in 2013.

Laboratory Response Network (LRN)

The KCDC and KNIH established the LRN in 2002 to maintain an early detection system for monitoring high-risk pathogens as bio-terror agents, which can be included in samples from patients or the environment.

LRN STRUCTURE

- Level C laboratory: only at the KNIH, Responsible for education and proficiency tests of level A and B laboratories. Definitive identification of clinical or environmental samples transported from level A and B laboratories.
- Level B laboratory: 17 Provincial Public Health and Environmental Research Institutes, one Chemical Biological Radiological Defense Command, one Armed Forces Medical Research Institute, and three National Quarantine Stations, The laboratories have either BL2+ or BL3 facilities and are capable of confirmatory testing for agents such as *Bacillus anthracis*.
- Level A laboratory: 10 National Quarantine Stations, 254 public health centers, 132 hospitals
- International LRN: The division of high-risk pathogen research of the KNIH joined as an international partner and member of U.S. CDC's LRN to strengthen the health security of the Republic of Korea and the world on December 12, 2013.

Bio-Resource

BIO-RESOURCE MANAGEMENT FOR PATHOGENS IN THE NATIONAL CULTURE COLLECTION FOR PATHOGENS (NCCP)

The NCCP, the centralized pathogen resource bank in Korea, has collected, preserved, and distributed pathogenic microorganisms and their information that has been clinically isolated from humans. The aim was to contribute to scientific communities by maintaining and distributing high-quality microbial resources useful for various research fields, particularly in medical science and laboratory healthcare. Until 2011, the NCCP was managed by the Division of High-risk Pathogen Research. The Pathogen Resource TF was independently separated from the division and launched highly specialized resources management in 2012. On March 19, 2013, the NCCP held the first symposium themed "Development and Utilization of Pathogen resources". More than 100 people in medical science and laboratory healthcare attended the symposium and shared information. The NCCP holding resources have been more than 10,000 strains, and the 2012 annual report of the NCCP, published on July 2013, disseminates related news. Many media sources have taken note of and mentioned the NCCP.

The NCCP has collected pathogen resources by collaborating with various entities. The regional banks are run by three university hospitals representing the Kyoungpook, Gyeongsang, and Chonbuk regions. The highly valuable characterized pathogens and primary isolates from patients were collected through two separate data banks. Other specific microbes such as *Mycobacterium* spp. and medical fungi were also collected by organizations conducting related projects. Thus, the NCCP collected 121 species and 196 strains of new pathogen resources during 2013 (Table 8).

TABLE 7. New pathogen resources of the National Culture Collection for Pathogens (NCCP) in 2013

Resources	Species
Bacteria	92
Fungi	44
Virus	35
Derivative materials	25
Total	196

TABLE 8. The distribution of National Culture Collection for Pathogens (NCCP) resources in 2013

Resources	Bacteria	Virus	Anti-serum & monoclonal Ab	Nucleic acids	Total
Distribution number(strains)	785	49	189	31	1,054

The NCCP now holds and manages 696 species and 4,902 strains, including viruses, bacteria, fungi, and the derivatives, and opened 424 species and 1,189 strains to the public for distribution through the NCCP website. The NCCP strengthens service for derivative resources. We developed 23 monoclonal antibody products and set up a quality control process. The NCCP has also developed several reference sets for national examination and education with domestic trends. It includes a reference set for supporting influenza virus research (15 Influenza virus strains, 15 rabbit and weasel antisera each, and 12 monoclonal antibodies) and an alternative set derived from domestic isolates according to the Korea Food Additive Codex experiment (three species and three strains).

A total of 105 species and 1,054 strains were distributed to relevant institutes and researchers in 2013 (Table 9). In addition, we identified a number of unidentified organisms and imprased long-term storage methods according to pathogen improved characteristics.

The NCCP has revised the NCCP work guidelines published in 2011 to improve the work process and has diversified management resources. NCCP members visited microbial culture collections such as the BIOTEC (National Center for Genetic Engineering and Biotechnology) culture collection, the TISTR (Thailand Institute of Scientific and Technological Research) culture collection, and the DMST (Department of Medical Science, Ministry of Public Health in Thailand) culture collection in Thailand in July 2013 and discussed ongoing exchanges between the culture collections and NCCP. A delegation from the TISTR culture collection visited the KCDC in November 2013 and toured the biosafety facilities and biobank building.

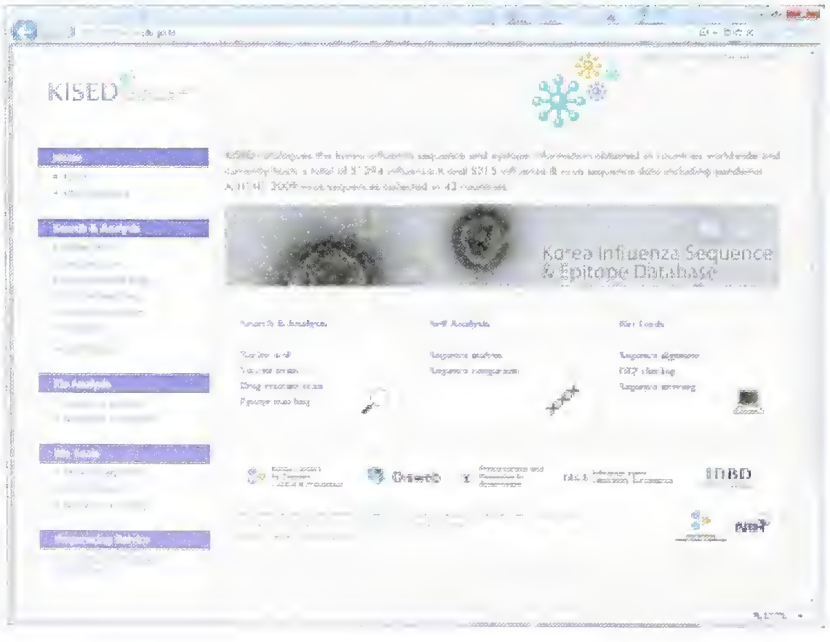


FIGURE 17. Main screen of the Korea Influenza Sequence & Epitope Database (KISED) home page (http://influenza.cdc.go.kr)

KOREA INFLUENZA SEQUENCE & EPITOPE DATABASE (KISED)

The Division of Influenza Virus has been running the KISED to make the best use of influenza virus genetic information. The KISED has been renewed with a modified web design and advanced web interfaces, and was opened on January 28, 2013. The KISED has collected 167,538 influenza type A and 14,014 influenza type B strains downloaded from National Center for Biotechnology Information Influenza Virus Resource, and genetic information of viruses isolated in Korea. The user can search a variety of web interfaces related to influenza virus genetic information such as drug-resistance analysis, epitope matching analysis, multi-alignment comparison analysis, and browse a geographical information system for worldwide influenza virus distribution. The KISED provides access for professionals and researchers to share information about influenza viruses. The KISED will become an international influenza database in the near future (Figure 17).

Accomplishments

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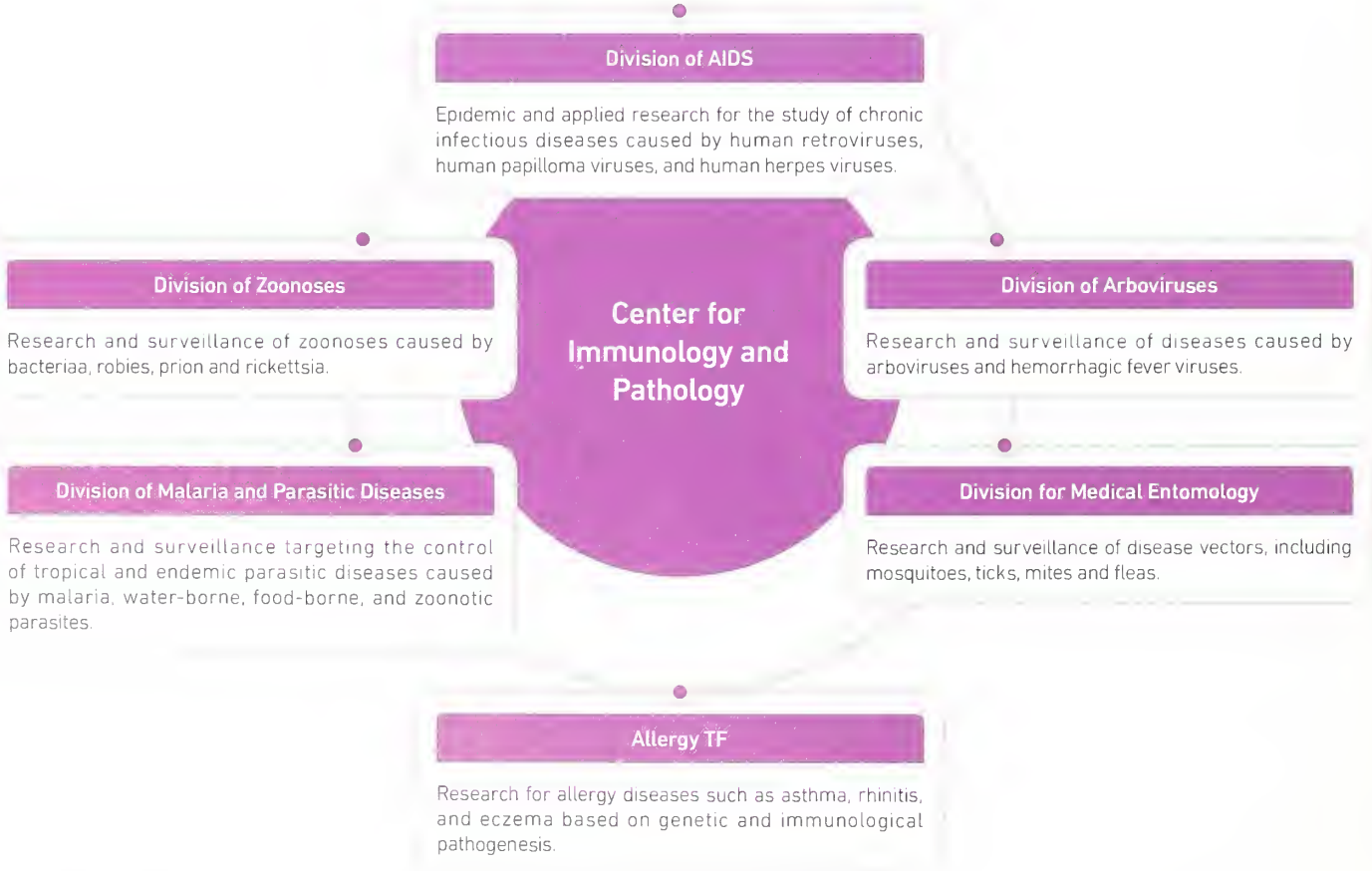
Center for Immunology and Pathology

The Center for Immunology and Pathology conducts basic and applied studies, with three primary goals: enhancing the national capacity for response to infectious diseases; actively carrying out research on climate change affecting environmental human health adaptation; and carrying out predictive research on newly emerging infectious diseases due to changes in lifestyle and ageing.

We contribute to reducing the high prevalence and huge burden of chronic infectious diseases, such as HIV and HPV, through research. Second, we reinforce the national response capacity and analyze new research demands in the fields of vector and vector-borne diseases, zoonoses, tropical diseases, and allergic diseases, which are predicted to have increasing prevalence with the onset and advancement of climate change. Finally, we prepare solutions to, and strategies for, emerging and reemerging parasitic diseases related to changes in lifestyle and in the environment.



We have developed: 1. a national reference laboratory to improve quality diagnostic assurance at national and civil laboratories, 2. a laboratory surveillance system to examine the prevalence of, and estimate changes in, infectious diseases, 3. cohorts to study the natural history of, and conduct translational research on, communicable chronic infectious diseases, 4. a database of virus sequences and pathogen proteomes for immunological and pathological research, 5. a collaboration network with domestic and international institutes.

National Surveillance of HIV, Vector-Borne, Parasitic and Zoonotic Diseases

HIV

Surveillance of HIV variants

We investigated the molecular epidemiological profiles of 1,316 HIV-infected Koreans diagnosed from 1985 to 2012. The most dominant HIV-1 subtype in Koreans was subtype B (Figure 1). Nine HIV-1 subtypes were examined as follows: subtype B (85.79%, n=1,129), subtype AE (5.47%, n=72), subtype A (5.01%, n=66), subtype G (2.27%, n=30), subtype C (0.98%, n=13), subtype CD (0.22%, n=3), subtype H (0.07%, n=1), subtype F (0.07%, n=1), subtype AGJK (0.07%, n=1).

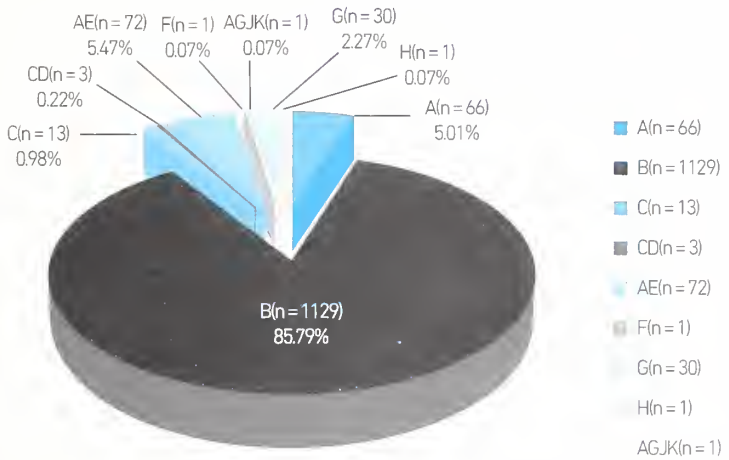


FIGURE 1. The distribution of HIV-1 subtypes in HIV-infected Koreans (1985–2012, n = 1,316).

Surveillance of resistant strains

The drug resistance trend in 1,125 newly diagnosed patients with HIV/AIDS between 1999 to 2013 is shown below. The predicted drug resistance related to protease inhibitors was lower than that of other antiretroviral drug classes, such as nucleoside reverse transcriptase inhibitors and non-nucleoside reverse transcriptase inhibitors in antiretroviral drug-naïve patients infected with HIV-1 for the last 15 years in South Korea. Total prevalence of drug resistance was about 6% (n=69). The prevalence of drug resistance has shown an increasing pattern in drug-naïve patients, since 2012 (Figure 2). We will continue to monitor the emergence of drug-resistant variants in a national survey. Furthermore, we must continuously investigate the effect of polymorphisms and the trend in drug-resistant virus transmission in drug-naïve patients.

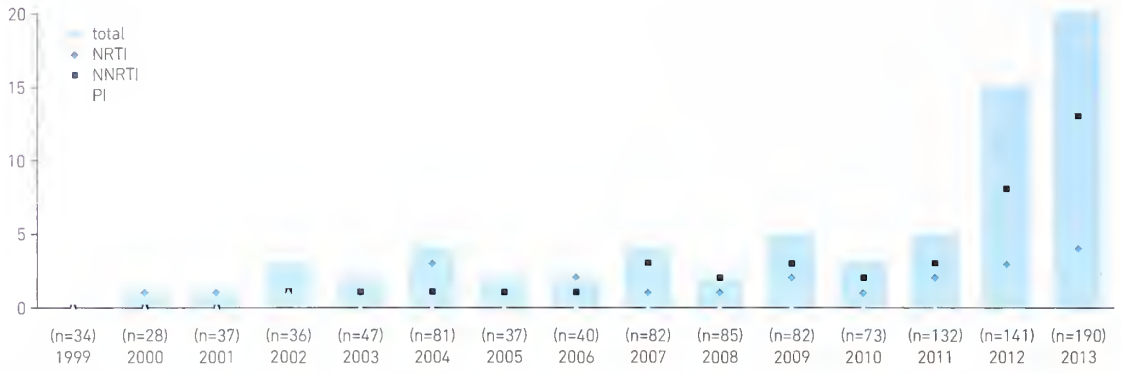


FIGURE 2. The prevalence of antiretroviral drug resistance based on drug class in newly diagnosed HIV drug naïve patients in South Korea, using genotypic drug resistance assay (n = 1,125). * NRTI, nucleoside reverse transcriptase inhibitors; NNRTI, non-nucleoside reverse transcriptase inhibitors; PI, protease inhibitors. * "SIR" Interpretation of the HIValg Comparison of Genotypic Resistance Algorithms from the Stanford DB (drug resistance levels: S, susceptible; I, intermediate; R, resistant).

Surveillance of HIV seroprevalence

We established a network of HIV-screening sites to estimate HIV seroprevalence nationwide. The HIV test results were gathered from screening sites, including public health centers, hospitals, and blood centers. HIV seroprevalence differed significantly by age group ($P < 0.001$) for male patients at colorectal hospitals, and was highest in

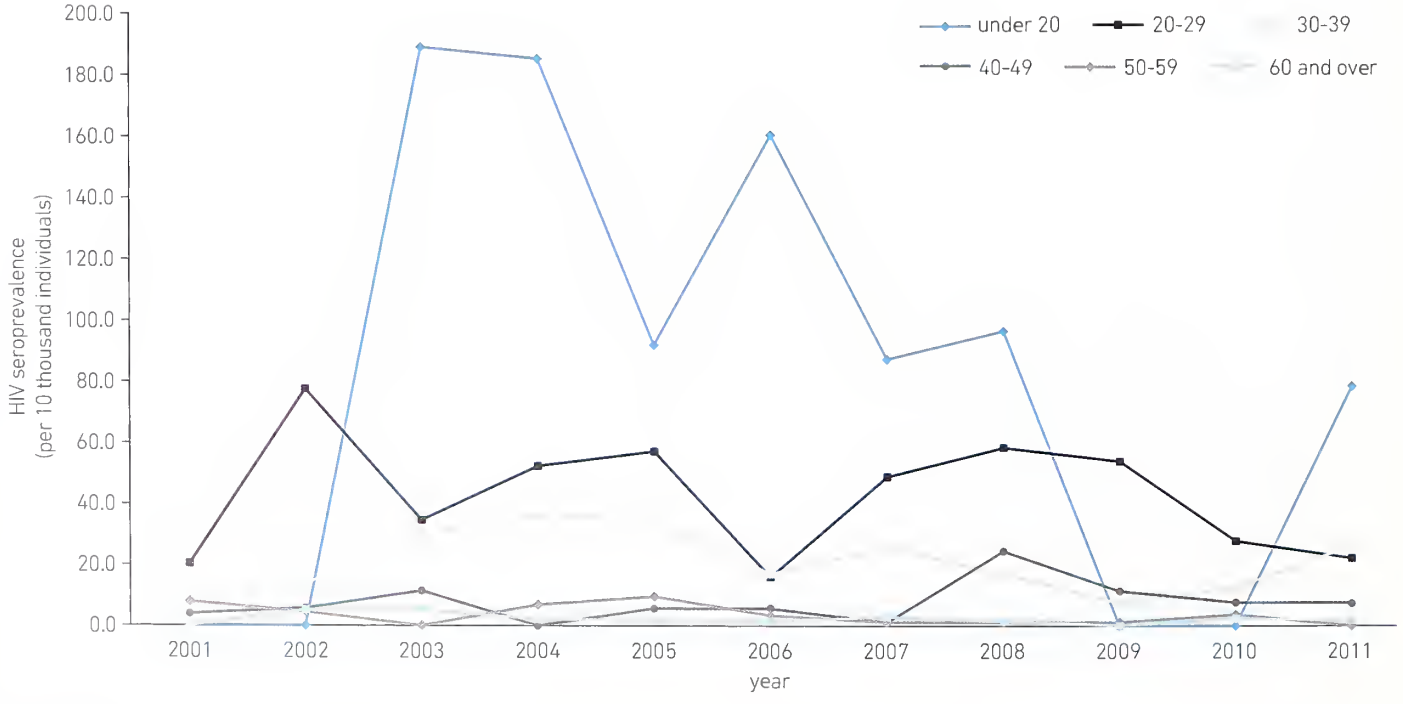


FIGURE 3. Trends in HIV seroprevalence by age among male patients with anal diseases, 2001–2011.

the group aged < 20 years for 11 years since 2001. HIV seroprevalence in patients with anal disease was 7.6/10,000–13.3/10,000 from 2007 to 2011, and that in patients with nonanal disease was 0–0.9/10,000. HIV seroprevalence in male patients with anal disease was significantly higher than that for other diseases (Figure 3).

VECTOR-BORNE DISEASES

Mosquito-borne diseases

Japanese encephalitis (JE) surveillance was performed using SYBR Green based real-time RT-PCR, which detects several flaviviruses, including dengue virus, West Nile virus, yellow fever virus, and JEV. A total of 1,794 mosquito pools were tested during the 2013 surveillance season (April–October) and 22 pools were flavivirus-positive. Further sequencing analysis revealed 10 cases were chaoyang viruses and 12 were JEVs. Chaoyang viruses were detected in only *Aedes vexans nipponii,* whereas JEV was detected in *Culex tritaneorhynchus* and *Cx. orientalis.* The JEV genotypes were types 1 and 5 based on a complete E gene analysis.

Seroprevalence in unvaccinated pigs was also investigated between July and October. A total of 2,320 sera were collected in slaughter houses in eight provinces. Among them, 292 sera (12.6%) were JEV sero-positive.

Scrub typhus

We developed a surveillance monitoring system and researched the mite and rodent *Orientia tsutsugamushi* infection rate. To survey *O. tsutsugamushi* infection rates in rodents and mites, rodents were captured using Sherman live traps, and chigger mites were harvested from rodents at 24 countrywide locations during 2011–2013. The 56-kDa outer membrane protein genes of *O. tsutsugamushi* were detected by nested PCR in blood or spleen from the captured rodents, and from 30 chigger mites per pool. The infection rates of each serotype in rodents from Boryeong (14.3%), Jangseong (11.1%), and Cheorwon (6.7%) in spring and Andong (33.3%), Muju (7.1%), Yesan (7.1%), and Sokcho (7.7%) in autumn were determined. The minimum positive rate (MPR) of mites in Uljin, Yeongju, and Yesan showed the highest infection rates of 2.2%, 1.4%, and 1.4% in spring, respectively. Moreover, the MPR of mites were high in Hwasung (3.7%), Chuncheon (3.2%), Pajoo(1.0%), and Cheorwon (0.8%) in autumn. In this study, Gyeonggi-do province showed a higher infection rate of mites than that of Gyeongsangnam-do, Jeollanam-do province in autumn. Because of the expected potential high risk, we must conduct continuous surveillance in Gyeonggi-do.

Severe fever with thrombocytopenia syndrome (SFTS)

SFTS is an emerging disease that causes high fever, thromobocytopenia, leukopenia, gastrointestinal symptoms (vomiting and diarrhea), hemorrhage and multi-organ dysfunction. *Haemaphysalis longicornis*, a major tick in Korea, is the principal vector for SFTSV.

To investigate the occurrence and prevalence of SFTSV in Korea, we collected ticks from nine provinces in South Korea during 2011 to 2012 and detected SFTSV by real-time RT-PCR. We collected 13,053 ticks, and *H. longicornis* (90.8%, 11,856/13,053) was the most abundant among them. The minimum infection rate (MIR) of SFTSV in *H. longicornis* was 0.5% (59 pools). SFTSV were detected in ticks of all stages, as the MIR was detected in larvae (2/350, 0.57%), nymphs (41/10436, 0.39%), males (2/221, 0.90%), and females (14/849, 1.65%). Viruses were detected in ticks collected from April to September. A higher MIR of the virus was observed in ticks from the southern part of Korea. We amplified partial genes of both the M and S segment from a sample and analyzed the nucleotide sequence of the genes which showed 93–98% homology to Chinese and Japanese strains.

We confirmed the existence of SFTSV in ticks for the first time in South Korea. The SFTSV prevalence data are particularly important for increasing awareness of SFTS in South Korea.

VECTOR SURVEILLANCE

Scrub typhus vector surveillance

We are currently performing the second periodic surveillance (2011–2013) at 24 localities in Korea. Periodic surveillance of trombiculid mites was conducted by collecting wild small mammals twice per year (spring and autumn) in four localities (Paju, Hwaseong, Cherowon, and Chuncheon) in 2013 to estimate the nationwide distribution

of scrub typhus vectors. From 135 trapped rodents, 26,279 chigger mites, representing four genera and 14 species, were collected, and their chigger index (C.I.) was 194.7. The predominant species were *Leptotrombidium pallidum* (58.1%), followed by *Neotrombicula tamiyai* (9.3%), and *N. kwangneungensis* (8.6%).

Chigger mite surveillance was conducted to clarify the seasonal distribution of scrub typhus vectors using new chigger mite collecting traps from four collection points in five regions (Okcheon, Yesan, Jinan Gurye, and Goryeong) from September to November 2013. Chigger mites first appeared during weeks 1 (Okcheon) and 4 (Goryeong) in September, and the density of chigger mites peaked at mid-November.

Japanese encephalitis (JE) vector surveillance

The population density of the JE vector mosquito *Culex tritaeniorhynchus* was monitored at 38 collection points in the Republic of Korea, and 792,560 mosquitoes, including 17 species in seven genera, were collected. *Anopheles sinensis* was the most common species followed by *Aedes vexans nipponii* and *Cx. tritaeniorhynchus*. The greatest number of *Cx. tritaeniorhynchus* (363) was trapped in the third week of August, and the density of vector mosquitoes was 87.1% less than that reported for the last 5 years.

Malaria vector surveillance

Mosquito surveillance uses mosquito population and malaria sporozoite infection data to assess the threat of human disease, identify high-risk geographic areas, assess the need for, and timing of, intervention events, monitor effectiveness, and improve prevention and control. The population density and malaria sporozoite infection rate of vector mosquitoes were determined at 38 collection sites in Incheon-si, Gyeonggi-do, and Gangwon-do near the Demilitarized Zone in the northern part of the Republic of Korea, using black light traps, from April to October. Adult anopheline mosquitoes were collected first in the second week of April, peaked in July, and declined in August and September, 2013. A *Plasmodium vivax* infection of vector mosquitoes occurred in July and August only in Gangwon-do.

SFTS vector surveillance

Ixodid ticks are major vectors of SFTSV and we surveyed the Ixodid tick population around sites of SFTS cases in 2013. Population surveillance of Ixodid ticks was conducted by two collecting methods (flagging and dry ice baited traps) at 14 localities from May to September. A total of 8,313 Ixodid ticks, representing three genera and four species, were collected. The predominant species were *Haemaphysalis longicornis* (99.0%), *H. flava* (0.9%), *Ixodes nipponensis* (0.1%), and one *Amblyomma testudinarium*.

PARASITIC DISEASE SURVEILLANCE AND CONTROL PROGRAM

The Parasitic Disease Control Program manages the overall planning and implementation of tropical and endemic parasitic diseases. The control program diagnoses and monitors food and water-borne parasites, including *Clonorchis sinensis, Metagonimus yokogawai, Cryptosporidium parvum, Giardia lamblia, Entamoeba histolytica*, and *Cyclospora cayatenensis* based on surveillance systems for both imported and domestic parasitic diseases. We strive to survey disease prevalence and to develop programs for decreasing *C. sinensis* infection rates of various parasites by genotyping. National recognition will be improved, and infectious diseases will be managed by eliminating the parasites. Thus, we pursue the management and development of diagnostic techniques for communicable diseases and pathogenic infections (North-East Asia Network Installation Project) and facilitate the prevention and management of water-borne protozoa through the diagnosis of diarrheal diseases.

Elimination Program for Clonorchiasis

Thirty-six counties were selected in five river basins located near or along the major rivers of Korea, with reference to the nationwide survey in 2012.

A total of 38,745 inhabitants (15,833 males and 22,912 females) were examined for intestinal parasites, and the study was undertaken from January to December, 2013. Of the 38,745 examined stool samples, 3,682 (9.5%) contained various intestinal parasite eggs, cysts, or larvae. The helminth eggs detected were 3,072 (7.9%) *C. sinensis*, 662 (1.7%) heterophyids, 75 (0.2%) *Trichuris trichiura*, 42 (0.1%) *Gymnopalloides seoi*, three (0.01%) *A. lumbricoides*, two (0.01%) *P. westermani*, two (0.01%) *Echinostoma spp*, one (0.003%) *D. latum*, and one (0.003%) hookworm. Protozoan cysts of *Giardia lamblia* were observed in two samples (0.01%).

The positive rates of clonorchiasis arising in sam-

ples from inhabitants surrounding river basins were 11.2% in Seomjingang, 9.7% in Geumgang, 7.3% in Nakdonggang, 2.1% in Yeongsangang, and 2.0% in Hangang. Of the 3,072 *C. sinensis* egg-positive cases, 55.4% were males.

The *C. sinensis* positive rate was highest in the 50–59 yr group (9.6%), followed by the 60–69 (8.5%), 40–49 (7.6%), and 70–79 yr (6.8%) group.

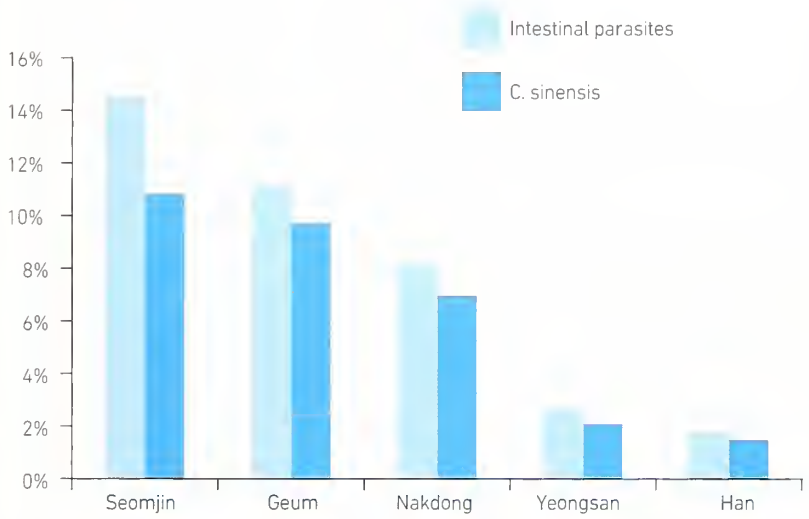


FIGURE 4. Prevalence of overall intestinal parasites and *Clonorchis sinensis* according to river basin.

Surveillance of *Plasmodium vivax* by merozoite surface protein-1 (MSP-1) gene-based genotyping

The prevalence of *P. vivax* subtypes was monitored by MSP-1 genotyping. In recent years (2007–2013), about half of the parasites identified in Korean patients belonged to the Sal I type (Sal-1: 48.6%; Belem: 19.6%; recombinant: 31.7%). Five allelic subtypes (S-a, S-b, S-c [Sal-1], B-1, and B-2 [Belem]) and a recombinant subtype have been identified since 2003. The subtype variation pattern has changed, as compared to that of a previous study. The proportion of B-1 (Belem type) increased from 13% (2010) to 24% (2013) and that of recombinant and Sal I type also increased. The B-2 and S-c types were not observed.

Enteropathogenic protozoa surveillance (Enter-Net Korea)

The 17 Research Institutes of Health and Environment and the KNIH have conducted surveys to detect enteropathogenic protozoa (*Cryptosporidium parvum*, *Giardia lamblia*, *Entamoeba histolytica* and *Cyclospora cayetanensis*). A total of 3,093 diarrheal fecal samples (Enter-Net Korea) were collected and analyzed by nested-PCR. Overall, 3.14% (97 cases) were positive. The positive rates of *C. parvum*, *G. lamblia*, *E. histolytica*, and *Cyclospora cayetanensis* were 0.74%, 0.87%, 0.04%, and 1.49%, respectively. The month with

TABLE 1. Positive cases of intestinal helminths and protozoa according to river basin and parasitic species

River basins	No. Exam.	No. of positive / Positive rates (%)															
		C. sinensis		Heterophids		*T. trichiura*		*G. seoi*		*A. lumbricoides*		*P. westermani*		*Echinostoma* spp.		*G. lamblia*		Others*	
Hangang	2,505	51	(2.0)	5	(0.2)	6	(0.2)	0	(0.0)	0	(0.0)	0	(0.0)	0	(0.0)	0	(0.0)	0	(0.0)
Geumgang	5,306	516	(9.7)	47	(0.9)	15	(0.3)	3	(0.1)	1	(0.0)	1	(0.0)	0	(0.0)	2	(0.0)	0	(0.0)
Yeonsangang	5,193	108	(2.1)	22	(0.4)	9	(0.2)	0	(0.0)	0	(0.0)	0	(0.0)	0	(0.0)	0	(0.0)	0	(0.0)
Seomjingang	13,255	1,481	(11.2)	488	(3.7)	19	(0.1)	14	(0.1)	1	(0.0)	1	(0.0)	2	(0.0)	0	(0.0)	3	(0.0)
Nakdonggang	12,486	916	(7.3)	100	(0.8)	26	(0.2)	25	(0.2)	1	(0.0)	0	(0.0)	0	(0.0)	0	(0.0)	0	(0.0)
Total	38,745	3,072	(7.9)	662	(1.7)	75	(0.2)	42	(0.1)	3	(0.0)	2	(0.0)	2	(0.0)	2	(0.0)	3	(0.0)

*Others: *D. latum*, *E. vermicularis*, hookworm

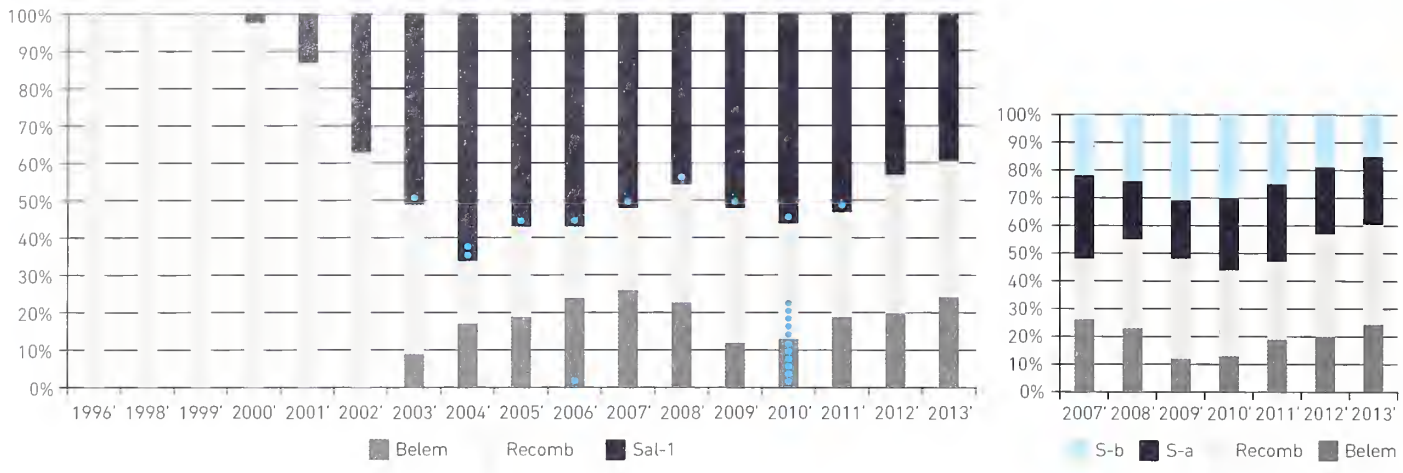


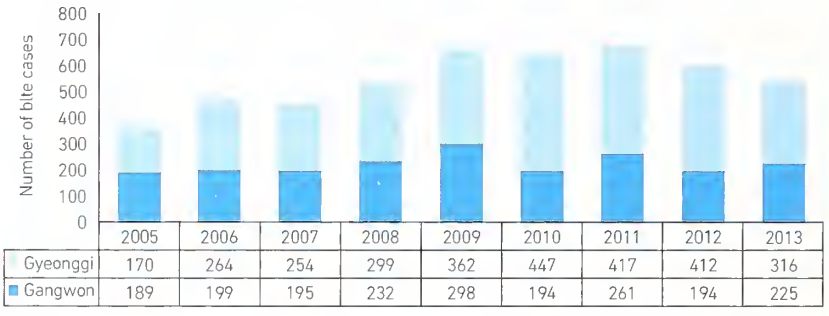
FIGURE 5. (a) *Plasmodium vivax* merozoite surface protein 1 (MSP-1) subtype comp osition in isolates obtained in Korea between 1996 and 2013. (b) The variation patterns of the MSP-1 gene subtype in Korean patients (2007–2013).

the highest positive rate was October (9.61%) and that with lowest positive rate was August (0.39%). In addition, the positive rates in males and females were 3.64% and 2.74%, respectively, and the highest (5.83%) prevalence was in the 21–30-year-old group, followed by the 11–20-year-old group (4.64%), the<1-year-old group (3.78%), and the > 60-year-old group (3.45%). *C. cayetanensis* infection was first detected in fecal samples in 2013,.

ZOONOTIC DISEASES

Rabies

A total of 553 animal bite cases in high-risk regions located in Gyeonggi (n=225), Gangwon (n=316) and other (n=12) provinces were reported in 2013. Most patients had been bitten in their hands or legs. The incidence was highest in adults in their 40s and 50s and the rates were not different from those of previous years. Dogs were the predominant animal biting humans, followed by cats. Some patients reported being bitten by raccoons, rodents (wild rats), bats, wild boars, and badgers. Most captured animals were observed for clinical signs of rabies for post-exposure prophylaxis of the patients. Complete or appropriate post-exposure prophylaxis was administered in about 40% of patients bitten by vaccinated animals.



	2005	2006	2007	2008	2009	2010	2011	2012	2013
Gyeonggi	170	264	254	299	362	447	417	412	316
Gangwon	189	199	195	232	298	194	261	194	225

	dog	cat	cow	racoom	wild animal	bat	unknown
2011	596	62	0	2	14	0	0
2012	480	74	0	11	26	1	2
2013	450	62	1	1	26	0	0

FIGURE 6. Animal bite cases in high-risk rabies regions from 2005 to 2013 (upper), and species identified in animal bite cases that occurred in high-risk rabies regions (lower)

Creutzfeldt–Jakob disease (CJD)

Laboratory CJD diagnoses were performed by screening CSF using the 14-3-3-protein detection method. Laboratory diagnostic criteria for CJD outlined by the WHO were used. The results of detecting the 14-3-3 protein were positive in 90 of 149 cases in 2013. In an analysis of human prion protein gene (*PRNP*) polymorphisms, 66 of 71 cases showed M129M in 2013. The characteristics of the *PRNP* analysis with respect to diseases related to CJD showed two cases of G142S, three cases of E219K, two cases of E200K, two cases of P68P, and three cases of M129V. Several correlation studies and developmental projects were performed for the *PRNP* gene. Epidemiological information for analyzing the potential genetic risk factors was collected, and a proteomic study to detect pre-mortem biomarkers was conducted. Clinicians immediately report patients with CJD because CJD/vCJD is the third national notifiable infectious disease group on the disease web statistic system (Korea CDC).

Brucellosis and Q fever

The seroprevalence of brucellosis and Q fever in each high-risk group for zoonoses is described in Table. We surveyed the seroprevalence of brucellosis and Q fever in livestock farmers and livestock hygiene controllers and inspectors in 2013. In 2013, the seroprevalence of brucellosis in livestock farmers (0.0%) showed the results which were lower than those of 2006. Also, the seroprevalence of Q fever in livestock farmers (3.7%) and livestock hygiene controllers (2.89%) showed the results which were similar or higher than those of 2006~2007. This shows more serosurveillance study of Q fever will be needed for investigating Q fever epidemiology.

TABLE 2. Seroprevalence of brucellosis and Q fever in high-risk groups.

Year	Profession	Brucellosis			Q fever		
		Seropositive (N)	Seroprevalence (%)	Number	Seropositive (N)	Seroprevalence (%)	Number
2006	Livestock farmers	15	0.23	6,721	28	1.04	2,690
2007	Livestock hygiene controllers	0	0.0	198	4	2.02	198
2013	Livestock farmers	0	0.0	860	32	3.72	860
	Livestock hygiene controllers	0	0.0	173	5	2.89	173
	Livestock hygiene inspectors	0	0.0	111	0	0	111

Research Projects

HIV/AIDS AND TUMOR VIRUSES

Since the first foreign HIV-infected patient was reported in Korea in 1985, the Division of AIDS has performed serological and immunological studies on AIDS-related opportunistic infections. The purpose of this laboratory was to establish an effective AIDStoP Project, which stands for Anti-HIV therapeutic challenges, Immunopathogenesis, Drug Resistance, Surveillance, and Papillomavirus study.

Anti-HIV therapeutic challenges

We used a yeast two-hybrid screening assay with full-length HIV-1 Tat as the bait, together with the human thymus cDNA library as prey, to identify the HIV-1 Tat binding partners. From 44 positive

clones resulting from nutritional selection, one clone contained a cDNA of 732bp (GenBank accession number NM_022731) encompassing the full-length open-reading frame of the NUCKS1 protein (Figure 7 A). Tat co-immunoprecipitated with NUCKS1 in cells expressing both Flag–Tat and V5–NUCKS1 but not in cells expressing either protein alone (Figure 7 B). Furthermore, endogenous NUCKS1 was immunoprecipitated by anti-Flag antibody in Flag–Tat-expressing HEK293 cells (Figure 7 C). HIV-1 LTR-driven luciferase and the NUCKS1 expression plasmid were introduced into HeLa cells in the presence or absence of the Tat expression plasmid to determine whether NUCKS1 has a functional role in Tat-induced transcriptional activation of the HIV-1 LTR. Although Tat markedly increased the LTR transcription, NUCKS1 expression increased this activity further by more than 2.3-fold (Figure 7D). Expression of increasing amounts of NUCKS1 increased Tat-mediated transcriptional activity on the LTR in a dose-dependent manner (Figure 7 E). NUCKS1 knockdown was performed with three siRNAs against NUCKS1, which effectively decreased the NUCKS1 expression level, to examine the effect of loss of function of NUCKS1 in Tat-mediated transcriptional activation of the HIV-1 LTR, (Figure 7 F). Tat-mediated LTR activation decreased significantly following NUCKS1 knockdown with these siRNAs, whereas NUCKS1 knockdown had no effect in the absence of Tat, which was consistent with showing no effect of NUCKS1 expression (Figure 7 G). These data demonstrate that NUCKS1 plays a crucial role in the Tat-mediated transcription on the HIV-1 LTR and suggest that Tat–NUCKS1 interaction may be required for this role.

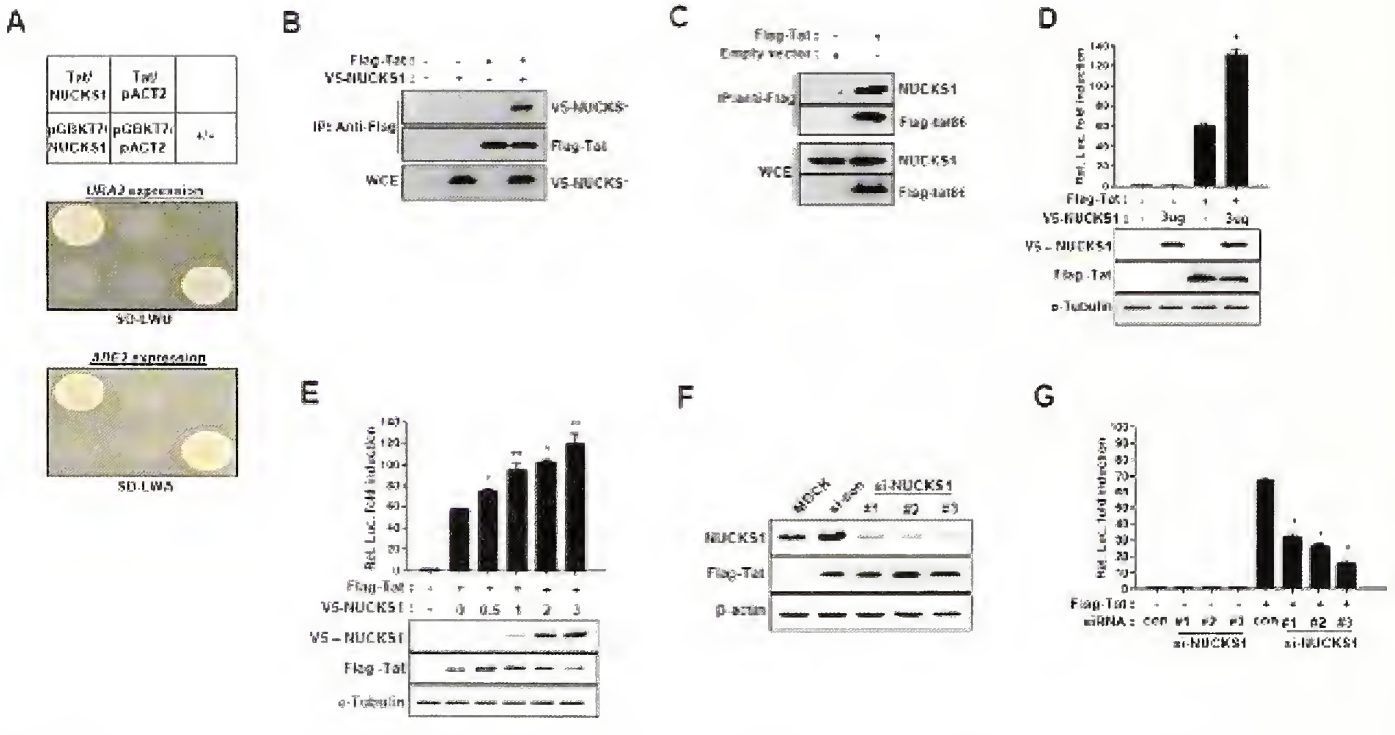


FIGURE 7. Interaction between HIV-1 Tat and NUCKS1. (A) The transformants were grown on an SD plate lacking adenosine and uracil for 48 h. Murine p53 and SV40 large T antigens were used as the positive control. The two yeast colonies panels show the interaction between Tat and NUCKS1. (B) Ectopically expressed Tat interacts with ectopically expressed NUCKS1 in HEK293 cells. (C) Co-immunoprecipitation assay between endogenous NUCKS1 and the ectopically expressed Tat protein. The interaction was assessed with Western blotting using anti-NUCKS1 or anti-Flag antibody. (D) Luciferase activity was measured and normalized to b-galactosidase activity 24 h after transfection. (E) Luciferase activity was assessed in HeLa cells 24 h after cotransfection of pGL3–LTR–Luc (200 ng), Flag–Tat (100 ng), and increasing amounts of V5–NUCKS1 (0, 0.5, 1, 2, and 3 mg) (F) NUCKS1 knockdown of was performed with three siRNAs against NUCKS1 mRNA. (G) NUCKS1-engaged Tat activity was assessed by the luciferase assay combined with the knockdown experiment.

TABLE 3. Comparison of plasma cytokine concentrations in HIV-1 negative plasma and the HIV-1 infected group. The median concentrations of cytokines/chemokines are shown and standard error is presented in parentheses to the right of each concentration value. (*$p < 0.05$, **$p < 0.005$, ***$p < 0.001$). [1] Comparison between HIV-1 negative and FS II-IV and [2] Comparison between HIV-1 negative and FS V-VI.

Function	Cytoline	HIV negative (n = 30)	HIV-1 infected group		p-value1)	p-value2)
			FS II-IV (n = 30)	FS V-VI (n = 30)		
Inflammatory	IL-6	1.30(0.30)	4.35(11.92)	2.27(10.86)	0.40	0.22
	IL-12(p40)	17.75(2.44)	46.50(4.06)	39.22(3.27)	0.0002***	0.0001***
	IL12(p70)	2.38(1.02)	1.06(1.29)	1.63(1.43)	0.58	0.66
	TNF-a	1.15(0.28)	0.42(0.54)	0.92(1.09)	0.95	0.07
	IFN-b	3.16(1.76)	9.21(10.77)	19.63(3.92)	0.41	0.03*
Chemokines	IP-10	59.68(10.39)	1045.58(97.79)	289.24(36.93)	0.0001***	0.0001***
	MIP1a	6.34(2.00)	10.82(1.63)	7.87(1.26)	0.04*	0.75
	MIP3a	11.80(2.44)	10.42(4.16)	22.96(11.69)	0.36	0.12
	MIP1b	512.37(119.25)	584.74(255.63)	543.78(615.97)	0.37	0.35
	RANTES	751.74(207.81)	1465.86(202.03)	1022.36(167.73)	0.02*	0.20
Anti-inflammatory Hematopoietic Adaptive	IL-10	0.46(0.26)	1.00(0.36)	0.85(0.95)	0.09	0.11
	IL-7	23.76(4.11)	32.86(3.32)	28.98(3.144)	0.07	0.47
	IFN-g	0.36(0.47)	1.39(1.13)	0.30(1.30)	0.06	0.15
	IL-2	0.90(0.69)	1.03(1.35)	1.62(1.60)	0.97	0.17
	IL-4	3.97(1.06)	1.15(0.63)	2.72(1.32)	0.68	0.75
	IL-5	2.68(0.89)	1.27(1.89)	3.21(2.97)	0.64	0.22
	IL-13	6.75(1.08)	1.89(0.86)	4.33(0.94)	0.0001***	0.24
	IL-15	13.83(4.06)	6.21(6.67)	14.33(9.04)	0.94	0.10

Immunopathogenesis

The levels of soluble factors in plasma samples grouped as early HIV-1 infection, chronic phase infection, and HIV-1 negative were measured by 18-plex immunoassay to compare the cytokine levels. Of the 18 factors measured, inflammatory cytokine IL-12p40 and the chemokines IP-10, MIP-1a, and RANTES increased significantly in plasma from the early HIV-1 infected group (FS II-IV) compared with those in HIV-1 negative plasma, whereas these factors were detected at lower levels in the chronically HIV-1 infected (FS V-VI) group than those in the early group (Table 3). In contrast, the anti-inflammatory cytokine IL-13 decreased significantly in plasma from the early HIV-1 infected group (FS II-IV) compared with that in HIV-1 negative plasma (Table 3).

Drug Resistance

The genotypic drug resistance assay has been the only method available to provide drug resistance-related information in South Korea since 1999. A phenotypic assay is a useful method to predict a patient's status related to antiretroviral drug resistance. To investigate patient-derived drug susceptibility to HIV-1 pseudoviruses, a phenotypic drug susceptibility assay using a modified single-round assay was established in 2006. Several group-derived pseudoviruses were investigated and analyzed focusing on a comparison between genotypic drug resistance and phenotypic drug susceptibility, including drug-naïve, treatment-experienced, and discordant patients. The phenotypic assay results provide incomplete information such as fold increases in the IC_{50}.

Nevertheless, the phenotypic assay is expected to help with better medical treatment, including information such as interpretation of mutual connections and cross-resistance among drug-resistant or polymorphic-related HIV variants.

Human papillomavirus (HPV) detection

Accurate human papillomavirus (HPV) typing is essential for evaluating and monitoring HPV vaccines in cervical cancer screening and in epidemiological surveys. In the present study, we constructed candidate reference materials comprising 15 targets (13 types of high-risk HPV, two types of low-risk HPV) and evaluated whether the candidate reference materials could be used as the reference for HPV detection and genotyping using quantitative real-time polymerase chain reaction. Standard curves for the wide linear range (10^1–$.10^6$ copies/μL) produced high correlation regression coefficient R^2 of 0.99. The reaction efficiencies were 96.3% to 101.2% for the standard curves, indicating highly efficient reactions. These results suggest that these reference materials may provide useful standards for standardizing quality assurance for different HPV-typing assays and for proficiency testing in diagnostic laboratories.

RICKETTSIAL DISEASES AND ZOONOSES

Scrub typhus

STUDIES ON IMMUNOPROTECTION AGAINST *ORIENTIA TSUTSUGAMUSHI* INFECTION

To develop *O. tustusgamushi* vaccine based on

TABLE 4. TaqMan probe assay performance for 15 different HPV genotypes

Genotype	Ct value by copy number[a]						Slope	R^2	% efficiency
	10^1	10^2	10^3	10^4	10^5	10^6			
HPV-6	35.95	32.58	28.94	25.61	22.21	18.92	–3.36	0.999	99.2
HPV-11	34.76	31.96	28.77	25.39	21.97	19.1	–3.263	0.999	101.25
HPV-16	35.87	31.84	28.32	24.89	21.49	18.15	–3.36	0.999	99.2
HPV-18	35.96	33.02	29.52	25.96	22.49	19.63	–3.263	0.999	101.25
HPV-31	35.61	31.77	28.39	24.98	21.7	18.4	–3.326	0.999	99.95
HPV-33	36.02	32.49	29.03	25.56	21.91	18.56	–3.498	0.999	96.55
HPV-39	35.89	33.43	30.12	26.75	23.12	19.74	–3.436	0.997	97.7
HPV-45	34.87	31.87	28.91	25.54	22.21	18.73	–3.402	0.998	98.4
HPV-51	34.75	31.7	28.41	25.03	21.66	18.28	–3.375	0.999	98.9
HPV-52	33.49	30.34	27.07	23.68	20.07	16.84	–3.433	0.999	97.8
HPV-56	36.61	33.6	30.06	26.76	23.37	19.94	–3.401	0.999	98.4
HPV-58	34.75	32.45	28.8	25.52	22.06	18.71	–3.422	0.997	98
HPV-59	37.12	32.2	28.56	25.5	21.38	18.27	–3.512	0.995	96.3
HPV-66	35.93	33.48	30.07	26.6	23.14	19.76	–3.436	0.997	97.7
HPV-68b	35.28	32.3	29.09	25.65	22.23	18.99	–3.374	0.999	98.95

[a] Ct, cycle threshold values.

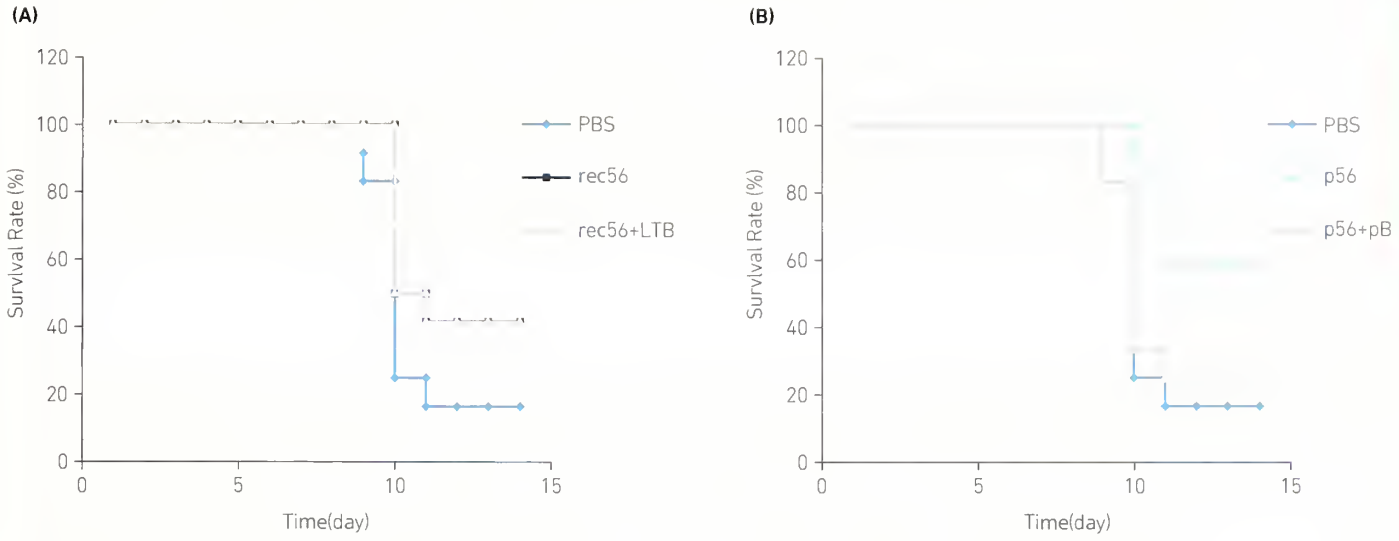


FIGURE 8. Evaluation of protection conferred on immunized mice against a live *Orientia tsutsugamushi* Boryong challenge. (A) Intranasal immunization with rec56. (B) Intramuscular immunization with p56. Survival rate was calculated as the ratio of living mice to total mice challenged in a group.

TSA56, which is a major outer membrane protein of *O. tsutsugamushi*, we designed recombinant protein (rec56) and TSA56 plasmid DNA (p56) vaccines. Intranasal (I.N.) immunization of rec56 or rec56 with the heat-labile enterotoxin B subunit from *E. coli* (LTB) induced a higher level of TSA56-specific immunoglobulins, as compared to that of intramuscular (I.M.) immunization of p56 or p56 with pBOOST2-samIRF7/3 (pB). However I.M. immunization of p56 or p56 with pB induced a strong cellular immune response to TSA56, as demonstrated in a splenic cell proliferation assay. Relatively strong protection against a homologous challenge with the *O. tsutsugamushi* Boryong strain was observed in mice immunized with p56. We anticipate that these vaccines could be used to study the immune mechanism of protection against scrub typhus and to design a polyvalent recombinant protein or DNA vaccine.

ANALYSIS OF *O. TSUTSUGAMUSHI* MAJOR ANTIGENIC PROTEIN EPITOPES CAUSING SCRUB TYPHUS

We analyzed 47 and 56 kDa outer membrane proteins as antigen epitopes, and several peptide epitope clusters were identified in these *O. tsutsugamushi* Boryong strain proteins. We found that the most reactive epitope regions of the 47 kDa and 56 kDa proteins induced antibody responses in sera from patients with scrub typhus. As a result, the 56 kDa protein (amino acid residues 49–99, 153–239, and 268–323) were more reactive to patient sera than those of the 47 kDa protein (amino acid residues 41–59, 177–208, 361–383, and 421–436). Other immunogenic responses to the 47 kDa and 56 kDa peptides occurred in sera from patients with scrub typhus. The 56 kDa peptide was more reactive to patient sera than that of the 47 kDa peptide, but the 47 kDa peptide produced similar responses among patient sera. These epitopes are expected to be used as peptide antigens for vaccines and as diagnostic candidates.

Q fever

We set up a complementary locked primer-based real-time PCR method and adapted recombinant proteins as ELISA antigen candidates, such as the outer membrane proteins of *C. burnetii*, to develop more sensitive and accurate diagnostic tools for detecting Q fever. We isolated *C. burnetii* using animal models and cell lines for Q fever and obtained a candidate of Q fever Korean isolate. A Q fever antibody for an ELISA was developed, but additional modification is needed for it to be

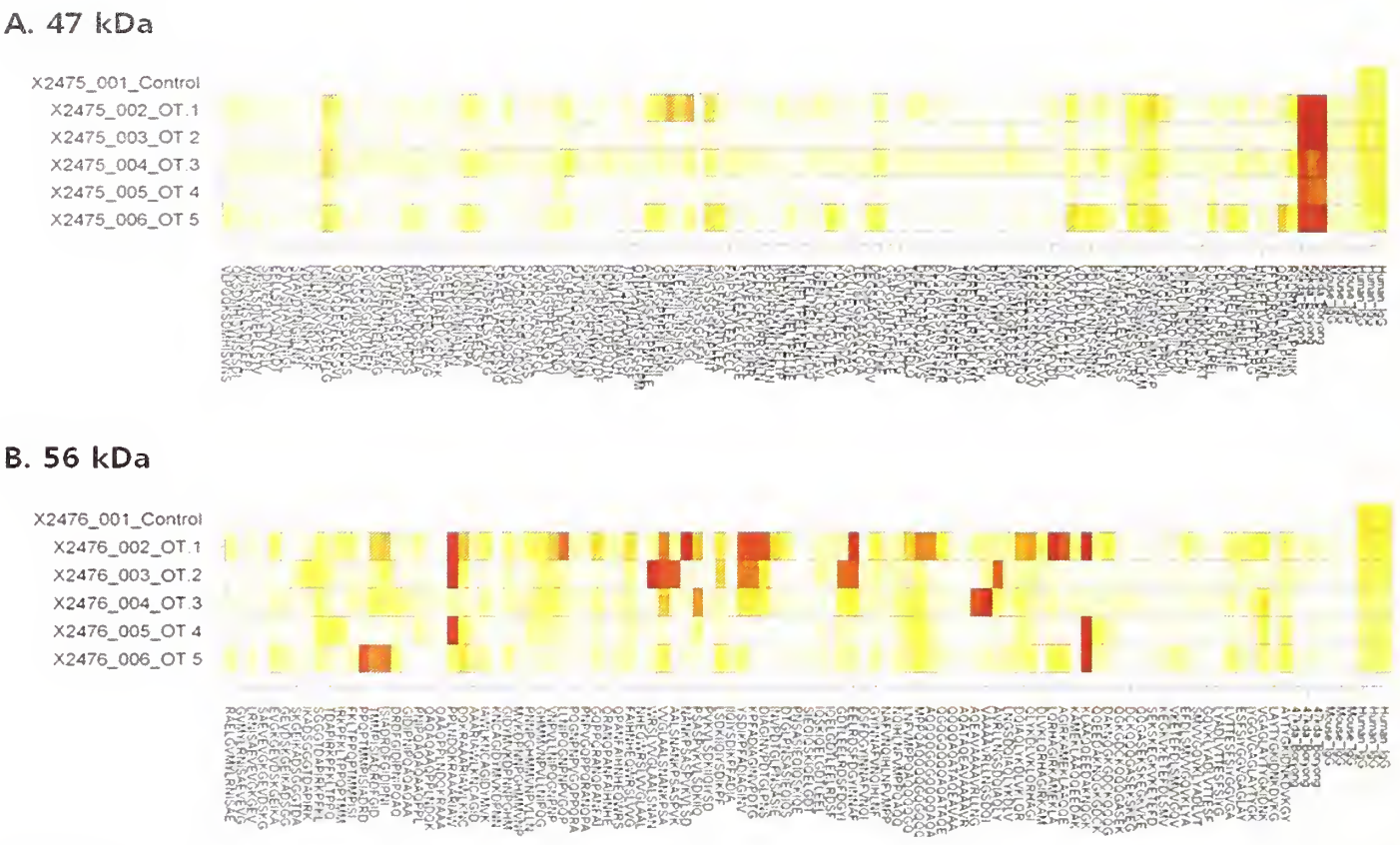


FIGURE 9. The results of epitope analysis from the 47 kDa and 56 kDa proteins of the *Orientia* tsutsugamushi Boryong strain using sera from five patients with scrub typhus.

used in a commercial kit. Continuous monitoring is scheduled to isolate *C. burnetii* originating from human source in Korea.

Tularemia

To analysis expression patterns and genes selection of virulence factors to *Francisella tularensis* Pohang, we carried out *in vitro* cell-infection A549, HepG2, J774A.1, and Microarray with a Pohang isolate, the *F. tularensis* live vaccine strain (LVS), and *F. philomiragia ATCC 25015*. Among the 1,754 genes such as *capA*, *iglA* and *wbtA*, which are 2-time over or down expressed virulence factors, the selected 51 gene were confirmed with conventional PCR. The thirty two genes were known virulence factors, 19 genes were over-expressed, function unknown gene.

Rabies

The rabies virus (RV) is a member of the family Rhabdoviridae, genus *Lyssavirus*. The rabies virus genome is approximately 12 kb, and is encoded with five structural proteins, including a nucleoprotein (N), a phosphoprotein (P), a matrix protein (M), a glycoprotein (G), and RNA-dependent RNA polymerase (L). Consequently, reverse genetic systems for RV require constructing full-length genomic cDNA plasmids and three helper plasmids.

We established a reverse genetic system for chimeric KGH (Korean rabies virus isolate) and the RC-HL strain. The full-length cDNA of pKGH/RC-HL was placed between HamRz and HdvRz to generate exact ends at both sides of the viral antigenome [Figure XX]. We cloned the five fragments separately in a pCI vector, added a hammerhead ribozyme

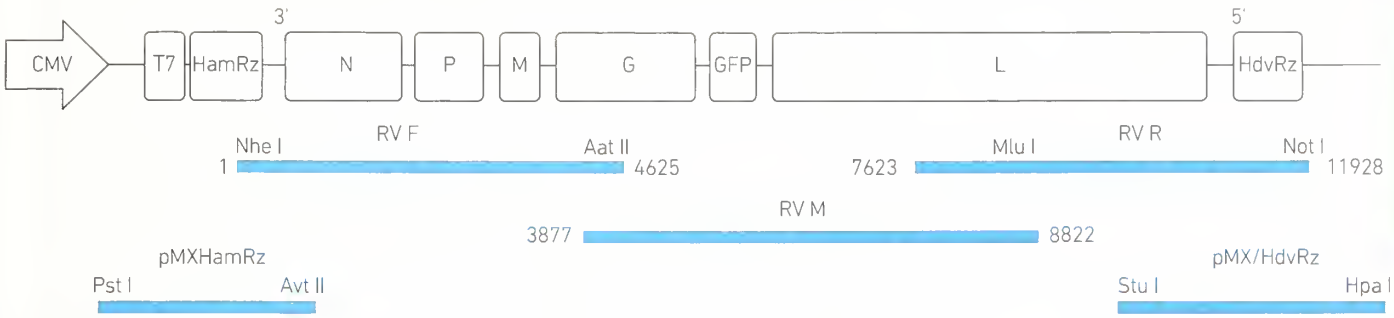


FIGURE 10. Strategy for assembling the full-length genome plasmids.

(HamRz) at the 5'-end and hepatitis delta ribozyme (HdvRz) at the 3'-end. Additionally, the gene expressing GFP was incorporated. BHKT7-9 cells were transfected with the pKGH/RC-HL plasmid along with the pCI/KGHN, pCI/KGHP and pCI/KGHL plasmids encoding the RNP complex protein to recover the recombinant RV from the cloned cDNA. The RV antigen was detected 7 days after transfection. The recovered recombinant RV was confirmed by indirect fluorescence assay and the GFP gene was expressed in the cytoplasm of the transfected cells [Figure 10]. Synthetic RVs recovered from the culture supernatant of the transfected cells 24 h post-transfection exceeded 1 x 10^7 focus-forming units /ml. The reverse genetics system for RV will be used in research fields related to etiology and vaccine development.

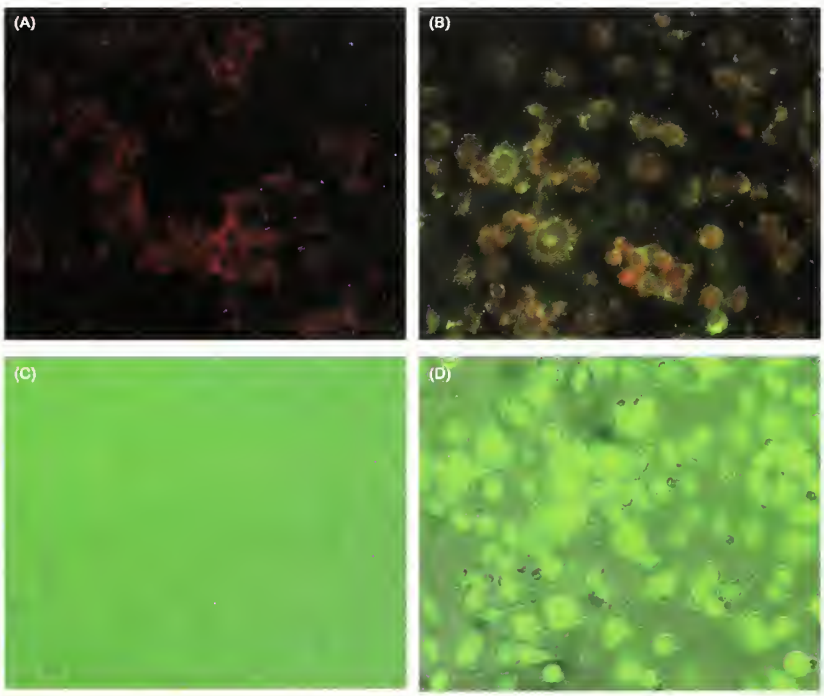


FIGURE 11. Rabies virus antigens and green fluorescent protein expression observed in Neuro-2A cells after transfection of the pKGH/RC-HL genome and helper plasmids. An indirect fluorescence antibody test using an anti-N protein monoclonal antibody was performed (A and B). Neuro-2a cells were

CJD

Experimental transmission of human prions to animals plays a major role in investigations of human prion diseases, because infectivity, the most important feature of prions (PrP), cannot be evaluated precisely *in vitro*. We produced six lines of C57BL/6 transgenic mice that express the human prion gene. The *PRNP* were constructed using the pPrPpE1/E2,3sal expression vector. Genes were inserted by nuclear injection into a one-cell embryo of a DNA segment containing the human PrP ORF, which was placed under control of a mouse prion gene promoter. After mating with PrP null mice, no detectable behavioral abnormalities in offspring were observed during development or during passage. The presence of exogenous human PrP in the brain and spleen of offspring (F3 or F4) was confirmed by RT-PCR, Western blotting, and immunohistochemistry. These four established transgenic lines will be used as animal models to understand the physiological function and the mechanistic roles in transmission of various human PrP.

ARBOVIRAL DISEASES

Anti-apoptotic mechanism of Hantaan virus

The nucleocapsid (N) protein of Hantaan virus (HTNV), which causes hemorrhagic fever with renal syndrome, is the most abundantly expressed protein in HTNV-infected cells. The influence of the N protein on HTNV-infected cells has not been studied. Some data indicate that the HTNV N protein inhibits apoptosis. To elucidate the regulation of apoptosis by the N protein, expression of the p53 protein, one of the key molecules involved in apoptosis, was assessed in the presence of the N protein in A549 and Hela cells. The amount of p53 increased by drug treatment was reduced when cells were infected with HTNV or transfected with an HTNV N protein expression vector. *p53* expression did not decrease in N protein-overexpressed cells when the cells were treated with a proteasome inhibitor (MG132) or an MDM2 antagonist (Nutlin-3). We concluded that the HTNV N protein ubiquitinates and degrades p53 MDM2-dependently. we studied downregulation of p53 expression through a post-translational mechanism, MDM2-dependent ubiquitination, and degradation by the HTNV N protein. These results indicate that N protein-dependent p53 degradation through the ubiquitin proteasome system is an HTNV anti-apoptotic mechanism.

Generation of flavivirus viral-like particles as vaccine candidates

Japanese encephalitis virus (JEV), Dengue virus (DENV), West Nile virus (WNV), Tick-borne encephalitis virus (TBEV), and Yellow fever virus (YFV) belong to the family *Flaviviridae* and are important mosquito-borne human pathogens. Virus-like particles (VLPs) of flaviviruses generated from the premembrane (PrM) and envelope (E) genes are promising vaccine candidates. Therefore, we established VLP expression of JEV, DENV, WNV, TBEV, and YFV in *Drosophila* cell lines. The PrM and E protein coding sequences of JEV, DENV, WNV, TBEV, and YFV were cloned into the *Drosophila* expression vector (pMT/BiP/V5/His vector), and *Drosophila* S2 cells were transfected with these plasmids. Cells expressing the JEV, DENV, WNV, and TBEV PrM and E proteins were confirmed by Western blot analysis using monoclonal antibodies directed against the flavivirus E protein. The VLP particles of JEV, DENV, WNV, TBEV, and YFV were also detected by electron microscopy. We carried out *in vivo* studies of the flavivirus VLPs to investigate the immune responses of the VLPs as vaccine candidates. The mice were tested for a humoral immune response using the plaque reduction neutralization test. The results revealed that monovalent VLPs of each flavivirus induced high–titer neutralizing antibodies in the mice. Moreover, bivalent VLPs of DENV and YFV efficiently enhanced neutralizing antibodies against flaviviruses. Further *in vivo* viral challenge experiments will be conducted to determine the protective efficacy of the flavivirus vaccine candidates.

Prevalence of JEV neutralizing antibodies in the adult population

We have conducted a JEV sero-survey in the adult population since 2011 to address the age shift from child to adult of patients with JE. In 2013, we tested 264 serum samples retrieved from the National Biobank of Korea. The samples were collected

TABLE 5. Prevalence of neutralizing antibodies to Japanese encephalitis virus among the adult population

Province	Positive rate (%) among age group				
	30s	40s	50s	60s	Total
Seoul	100(25/25)	100(25/25)	100(25/25)	96.4(24/25)	99(99/100)
Gyeonggi-do	100(25/25)	100(25/25)	100(25/25)	100(25/25)	100(100/100)
Jeju-do	100(15/15)	100(21/21)	100(15/15)	100(13/13)	100(64/64)
Total	100(65/65)	100(71/71)	100(65/65)	98.4(62/63)	99.6(263/264)

from subjects living in Seoul, Gyeonggi, and Jeju provinces during the Fifth Korea National Health and Nutritional Examination Survey (2010). The results showed that 99.6% (263/264) had neutralizing antibodies > 1:10 against JEV, indicating that most people obtained protective antibodies either by vaccination or natural infection (Table XX).

Dengue fever laboratory diagnosis by detecting IgM, the nonstructural 1 (NS1) antigen, and viral RNA

Dengue fever has a large global disease burden. Immunoglobulin M (IgM) and dengue virus (DENV) nucleic acid detection as well as virus isolation have been used as laboratory diagnostic assays for dengue fever. Detection of the NS1 antigen has recently provided a useful diagnostic tool. Determining the duration and intensity of the immune response and the first detection time of DENV antibodies and antigens are influenced by sensitivity and specificity of the assays and the specimen collection time. Specimens are collected during a wide time range of an infection episode and paired sera are usually not available. Therefore, a dengue diagnostic algorithm must be developed to support the likelihood of a correct laboratory diagnosis. In the present study, the dengue positive rates of an IgM capture ELISA, an NS1 antigen ELISA, and nested RT-PCR were analyzed in 602 patients with suspected dengue fever who had traveled to dengue endemic countries. The IgM ELISA, NS1 antigen ELISA, and RT-PCR showed positive rates of 38.4%, 47.3% and 17.6%, respectively. The positive rate increased by combining the assays to 54.0% in the IgM ELISA and NS1 antigen ELISA, to 40.7% in the IgM ELISA and RT-PCR, to 36.9% in the NS1 antigen ELISA and RT-PCR, and to 54.5% in the three assays. The results suggest that the NS1 antigen assay combined with the IgM antibody capture ELISA could increase sensitivity of the assays and help accelerate laboratory diagnoses of dengue fever.

Development of molecular diagnostic method for the NP gene of filoviruses

Ebola virus and Marburg virus belong to *Filoviridae* and are causative agents of severe hemorrhagic fever with high mortality rates in human and non-human primates. No licensed vaccines or therapeutics are avaiable to treat these viruses. Rapid identification of the virus is required to prevent spread of infection. Therefore, we developed a one-step real-time RT-PCR to rapidly detect five Ebola viruses and 2 Marburg viruses, targeting the NP gene of the viral genome and examined their sensitivities and specificities. The cDNA inserts encoding the NP gene of the filoviruses were synthesized and inserted into a vector. The RNA of the virus that was transcribed *in vitro* was used as a template. The limit of detection for this assay was 5–50 fg/μl RNA. The assay was highly specific for each filovirus and no cross-reactivity with other filoviruses was observed. Based on these data, the one-step real-time RT-PCR could be useful for detecting filoviruses.

MALARIA AND OTHER PARASITIC DISEASES

Immune regulation of allergic disease using *C. sinensis*-derived products

Our previous study (Biochem Biophys Res Commun, 2011, 407: 793–800) demonstrated that *C. sinensis*-derived crude antigens suppress development of allergic responses. We hypothesized that *C. sinensis*-derived products may directly interfere with either the allergic response or the allergen-specific Th2 response. Therefore, we identified *C. sinensis*-derived antigens that exhibit a potent suppressive effect on allergic inflammation to understand the mechanisms related to the helminth-mediated anti-allergic effect.

By predicting the antigenic proteins based on the *C. sinensis* expressed sequence tags database, we identified and sequenced a multitude of antigen candidates that bound to sera from patients with *C. sinensis* infection. The anti-allergic effects of these antigenic proteins were determined using rat basophilic leukemia (RBL)-2H3 cells. One candidate, the *C. sinensis* venom allergen-like proteins (CsVALs), significantly inhibited β-hexosaminidase release from RBL-2H3 cells. VAL proteins are expressed in many trematodes and nematodes. However, the molecular functions of these proteins remain unclear. However, a recent study of SmVALs in *S. mansoni* suggested that these proteins may play critical roles as immunomodulators in the host-parasite interaction. Our previous study (Biochem Biophys Res Commun, 2014, 445(3): 549–55) identified the anti-allergic effect of the CsVAL peptide using a mouse model of oxazolone-induced contact hypersensitivity *in vivo* and a mast cell line *in vitro*.

We investigated the prophylactic effect of immunizing with CsVAL using a mouse model of

ovalbumin (OVA)-induced allergic rhinitis to identify the appropriate effect of CsVAL on suppressing the allergic hyper-inflammatory response. In the current study, we explored the inhibitory activities of the CsVAL peptides on the development of severe nasal mucosal inflammatory disease. In the OVA-induced AR model, we showed that there was notable improvement in suppression of eosinophil, mast cell, granulocyte, and $CD4^+$ effector T cell infiltration in mice that received a prophylactic treatment with the CsVAL peptide. However, immunization with the CsVAL peptide had no effect on the induction of $CD4^+$ $CD25^+$regulatory T cells or IL-10-producing regulatory B cells, and these cells are important for modulating the immune response. Moreover, the CsVAL peptide does not appear to inhibit production of OVA-specific IgE, IgG1, or proinflammatory cytokine in sera or nasal fluids. Our results suggest that the inhibitory activities of the CsVAL peptide with regard to the severe inflammatory response offer a new potential therapeutic approach for allergic diseases such as atopic dermatitis and allergic rhinitis.

Immune regulation of allergic disease inducing regulatory B cells with *Toxoplasma gondii* infection

Parasites employ various strategies to evade effective host immune systems that thwart infection. Although immune evasion has been selected to favor establishment of the parasite within the host, it is likely that some particular strategies that escape the immune response might paradoxically be beneficial for the hosts. In fact, modulating the immune system by infection with parasites is proposed to suppress allergic inflammation.

Regulatory B cells, regulatory T cells, and alternatively activated macrophages were identified as key components of the immune regulatory network functioning during parasite infections. These immune regulatory cells increase during parasite infection and may promote survival of parasites and influence unrelated immune-driven pathology such as allergic and autoimmune diseases.

T. gondii is a worldwide obligate intracellular protozoan parasite that infects humans and animals by congenital and postnatal routes. Toxoplasmosis is usually clinically asymptomatic in healthy individuals, but it can cause severe complications in pregnant women and immunocompromised patients.

We investigated whether experimental infection with *T. gondii* could induce immune regulatory cells, including IL-10 producing $CD1d^{high}CD5^+$ regulatory B cells (Bregs) and $CD4^+$ $CD25^+$ $Foxp3^+$ regulatory T cells (Tregs), and whether Breg induction was relevant to parasite immune escape during *T. gondii* infection. *In vivo* depletion studies demonstrated an important role for Bregs in *T. gondii* survival. Furthermore, adoptive transfer of Bregs isolated from T. gondii-infected mice enhanced cyst formation in the brains of *T. gondii*-infected recipient mice.

We also investigated whether the development of allergic atopic dermatitis (AD) could be suppressed by *T. gondii* infection in mice. *T. gondii* infection substantially suppressed the development of AD-like lesions induced by the allergen *Dermatophagoides farinae* in NC/Nga mice, a model of human AD. Collectively, these results indicate that induction of Breg and Treg during *T. gondii* infection is necessary for evasion of host immunity, and these cells could suppress allergic inflammation.

Analysis of immune activity of heat shock proteins (HSPs) and antigenicity of HSP-binding proteins in *C. sinensis*

HSPs are abundant intracellular molecules that recognize and bind to polypeptides and partially folded intermediates of proteins, preventing aggregation and misfolding. HSPs represent dominant antigens in many infectious diseases that induce strong adaptrue and innate immunity. The roles of bacterial and mammalian HSPs have been confirmed but HSPs of parasites, except protozoa, are unclear. We investigated whether HSPs of *C. sinensis*, a liver fluke, play a role as molecular chaperones by binding proteins, and represent the antigenicity and immunogenicity through the expression of recombinant proteins.

rCsHSP90 had antigenictiy in an immunoblot analysis, but rCsHSP70 did not, using sera from patients with clonorchiasis. rCsHSP70 and rCsHSP90 bound to various *C. sinensis* proteins through immunoprecipitation and mass analysis. rCsHSPs blocked aggregation of proteins during expression and purification of recombinant proteins. These results indicate that rCsHSPs play a role as molecular chaperones, thereby contributing to solubilize aggregated protein.

We carried out a series of immunological reaction analyses with endotoxin-free rCsHSPs, to determine immunogenicity. rCsHSP70 or rCsHSP90 induced dose-dependent upregulation

in the expression of co-stimulatory molecules CD40, CD80, and CD86 on mouse bone marrow dendritic cells (mBMDCs). When mBMDCs were incubated with rCsHSP70, MHC I and MHC II also increased dose-dependently. Pro-inflammatory cytokines (IL-1β, TNF-α, IL-6, and IL-12p70) secreted from mBMDCs after treatment with rCsHSP70 or rCsHSP90 increased. IL-10 expression was also slightly induced. The Th1 cytokine, IFN-g, increased in allogenic T cells incubated with mBMDCs treated with rCsHSP70 or rCsHSP90, but the Th2 cytokine, IL-4, was released at basal levels. Our results indicate that rCsHSPs induced the innate immune response by activating dendritic cells and increasing secretion of pro-inflammatory cytokines. Furthermore, rCsHSP70 and rCsHSP90 induced a predominant Th1 response. In particular, rCsHSP90 more strongly stimulated mBMDCs or T cells than rCsHSP70.

Discrimination and characterization of patients with vivax malaria

MSP-1, circumsporozoite protein (CSP), Duffy binding protein (DBP), and apical membrane antigen-1 (AMA-1) were cloned into an expression vector. The recombinant proteins were reacted with different patient groups (relapsed and re-infected and long and short incubation patients), and antigenicity of each patient group was compared. The immune response based on cytokine and chemokine profiling of the host group (patients with malaria) was also analyzed. The human IgG response was characterized in a pilot study. T-cell differentiation in patients with malaria was tried using several fresh blood samples. Differentiation based on genotyping was attempted by allele-specific PCR (ASP). ASP based on AMA-1 and DBP was tested in 2013. This ASP technique will be modified and completed in 2014.

Identification of the common helminth antigen by epitope analysis

The use of common (conserved and orthologous) epitopes is expected to provide broader detection across multiple helminth species than epitopes derived from variable genomic regions. Here we describe an *in silico* method for genome-wide identification of common epitope candidates for three classes, including cestodes (C), nematodes (N), and trematodes (T). Orthologous genes were clustered and the gene shaving close hits in free-living organisms were filtered. Consensus sequences and the best hit sequences extracted from the remaining helminth proteins were used for further analysis. Secretory proteins were analyzed in terms of secretion, localization, and transmembrane. Linear B-cell epitopes ≥ 10 in length were predicted using a combination of five epitope prediction tools. Among them, common epitopes were selected based on the conservation score. Finally, collective sets consisted of 300 common epitopes as follows: 10 CNT, 150 C, 45 N, and 95 T. This set is being tested using peptide arrays and sera from four representative patients. This strategy can be used for any class of pathogen and provides new insights for developing effective diagnostic molecules.

DISEASE VECTOR CONTROL AND TRANSMISSION

Development of molecular markers for vector identification

To classify trombiculid mites using molecular markers instead of morphological identification, we analyzed the sequences of the internal transcribed spacer (ITS) regions of five *Leptotrombidium* species and three *Neotrombicula* species of trombiculid mites by PCR amplification using common tick ITS primer sets. The universal forward and specific reverse primer sets were designed for each chigger mite species, and the field-collected samples were successfully identified using these primer sets.

Vector ecology of *Aedes albopictus*

Dispersal ability was estimated by using the mark-release-recapture method in Korea. Fluorescence marked *Ae. albopictus* were released and recaptured in four different habitats. The maximum distance was 41 m in urban areas and 75 m in suburban areas using sticky traps. The maximum distance was 185 m in urban areas and 90 m in suburban areas using BG-sentinel traps. The maximum distance of dispersal was 205 m and 150 m in airport areas. In conclusion, *Ae. albopictus* has a narrow dispersal range, as compared to that of other mosquitoes, a typical characteristic of genus *Aedes*, and their dispersal ability was greatly affected by various environmental factors. Based on this result, control of *Ae. albopictus* should be focused around parks, bushes, and forest boundaries in urban and suburban areas. Our results provide basic information for effective control of *Ae. albopictus* and overseas inflow of dengue fever.

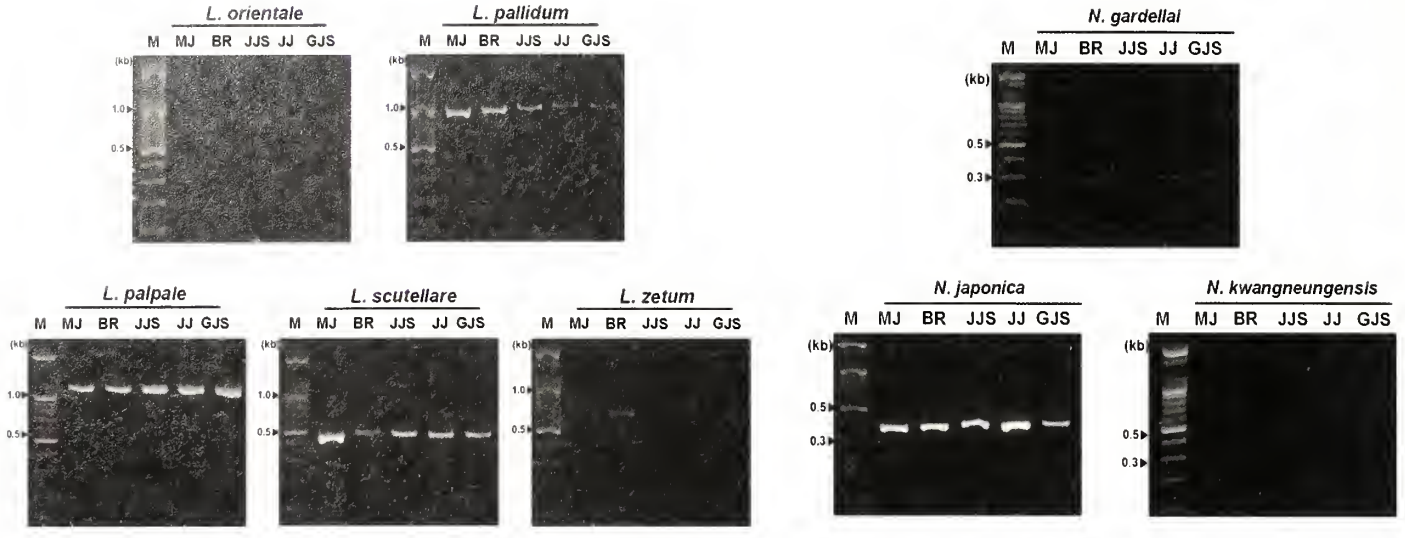


FIGURE 12. Molecular identification of field-collected chigger mites using species-specific primer sets.

Identifying the JE vector

JEV causes significant disease and is distributed throughout Asian countries. The virus is transmitted by *Culex tritaeniorhynchus*, which mainly breeds in rice paddies in Korea. However, one study reported that JEV genotype V was detected from a *Culex bitaeniorhynchus* mosquito in Korea. Moreover, the JE outbreak in 2010 was not correlated with the density of *C. tritaeniorhynchus*. Thus, we investigated the presence of other mosquito species that could transmit JEV as second or regional vectors. We selected five cities as mosquito-collecting locations where patients with JE have presented in the last 5 years and subdivided them into four collection sites according to mosquito habitat (cowshed, downtown area, forest, and swamp). Mosquitoes were caught using BG-Sentinel traps, CDC black-light traps, Fay–Prince traps, and gravid traps. Six JEV-positive pools were detected from *Culex orientalis* and *Culex pipiens* caught in Gangwon-do and Gyeonngi-do provinces. All detected JEVs were revealed as genotype V by phylogenetic analysis of the envelope gene (Fig.13). Our findings confirm that a new JEV genotype has been introduced to Korea and suggest that the two mosquito species may play a role in JEV transmission.

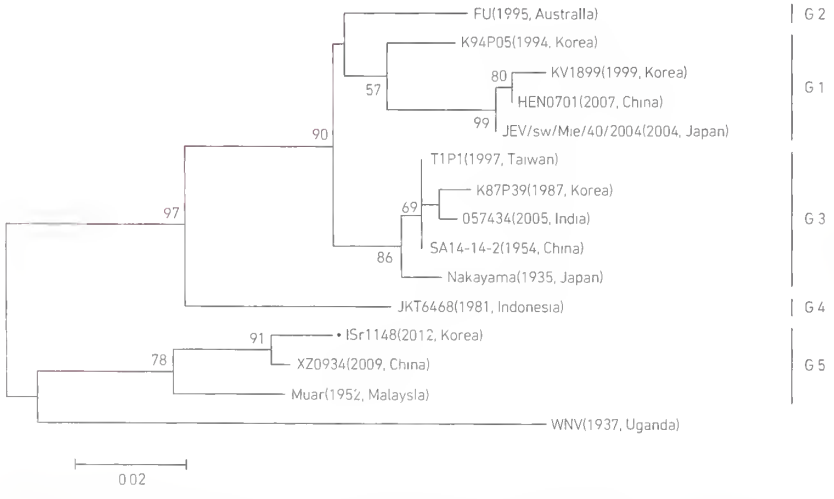


FIGURE 13. Phylogenetic analysis of Japanese encephalitis virus from Korea, 2013.

Vector control using *Wolbachia*

The intracellular endosymbiont bacterium *Wolbachia* is currently considered most abundant in arthropods. Many *Wolbachia* manipulate host reproductive systems, leading to male-killing, cytoplasmic incompatibility, parthenogenesis, and feminization of genetic males, with a large impact on arthropod host ecology and evolution. We investigated the distribution of *Wolbachia* infection in *Ae. albopictus* according to geographical

distribution. Our results indicate that *Ae. albopictus* are commonly infected with *Wolbachia*, and it is possible that *Wolbachia* may act as endosymbiont in *Ae. albopictus*, regardless of geographic region. Although a low infection rate of *Wolbachia* was detected, continuous surveys are needed to evaluate the *Wolbachia* strain within *Ae. albopictus* as a dengue fever vector.

Vector surveillance in preparation for climate and environmental changes

Six base centers were established in Honam, Yeongnam, Jeju, Chungcheong, Gangwon and Gyeonggi to effectively cope with climate change. Researchers in each area were educated on how to perform this project, and vector mosquitoes and mites were monitored for WNV, JEV, DENV, YFV and *O. tsutsugamushi*. Pathogens were detected in vectors. The population density of vectors was monitored at the six base centers. The results indicate that 466,737 mosquitoes, 24,659 mites, and 7,729 ticks were collected and classified from the six centers. Real-time PCR demonstrated JEV-positive results from one pool of vector mosquitoes collected from the Gangwon center. *O. tsutsugamushi* were detected from three base centers during the fourth week of April, the fifth week of May, the second week of October, and the third and forth weeks of November in the Honam area, the fourth week of September in the Jeju area, and in the fourth week of September, the second and fourth weeks of October, and the first week of November in the Gangwon area.

National vector control and surveillance

Vector-borne diseases are very difficult to prevent and control. Scrub typhus has caused over 8,000 reported human cases in 2013. Additionally, tick-borne diseases, such as Lyme disease, Japanese spotted fever, and SFTS have been reported in Korea. The Division of Medical Entomology of the National Institute of Health specializes in identification, ecology, prevention and control of vectors to respond to the challenges presented by vector-borne diseases. The national vector control and surveillance program conducts investigations on the biology, ecology, and control of arthropod disease vectors to develop new and/or modify existing measures for more effective prevention and control. It provides reference/diagnostic services for vector control; offers epidemic aid and epidemiology consultations upon request to local health departments; and provides technical expertise and assistance in professional training activities.

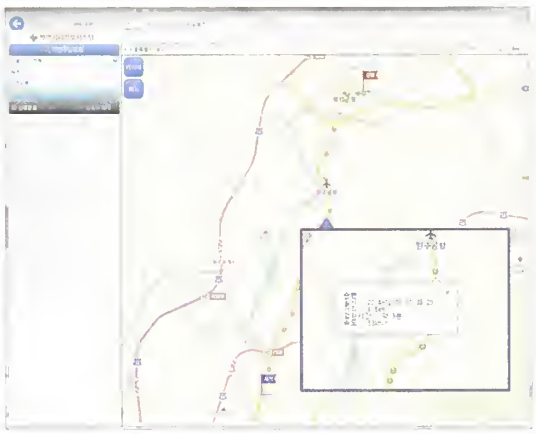


FIGURE 14. Personal computer showing the trace from an insecticide fogging vehicle used by the vector control system

The program activities are as follows.

- Construction and operation of a vector control system using GIS (Fig. 14)
- Publication of guidelines and a manual for vector control and management
- Monitoring of insecticide susceptibility and resistance
- Monitoring of outbreak pests
- Management of medical insect resources
- Education about vector control

Extension of Research Infrastructure

HIV/AIDS COHORT STUDY

The Korea HIV/AIDS cohort study was organized in 2006 as a multi-center cohort Qualified epidemiological and clinical information has been collected using standardized case report forms (CRFs), and the cohort staff has been periodically educated on use of the CRFs. As of 2013, 1,247 HIV-infected patients have been registered and followed every 6 months. In addition, 5,990 samples have been deposited in the Biobank and data have been collected in the KCDC database system. A community program has also been added to the database system to facilitate active communication among staff. HIV/AIDS research using cohort samples and data will be performed according to the publication policy and procedure of the cohort study.

HPV COHORT STUDY

The Korea HPV cohort study was established as a large prospective multicenter (five hospitals) study in September 2009. Women who are both HPV-positive with either atypical squamous cells of undetermined significance or low-grade squamous intraepithelial lesions of the cervix ware enrolled. The goals of this cohort study were to: 1) study the epidemiology of persistent HPV infection, 2) investigate whether persistent HPV infection increases the risk of low-grade and high-grade cervical lesions, and 3) research genetic and immunological determinants of persistent HPV infection. Samples and clinical data have been deposited at the KCDC to provide for HPV research in the future.

HEPATITIS C VIRUS (HCV) COHORT STUDY

The Korea HCV cohort was established with five university hospitals in January 2007. This prospective and multicenter cohort enrolled 1,746 patients who were anti-HCV antibody-positive and who completed a questionnaire related to risk factors for the disease, focusing on HCV infection in 2013. As of the end of 2013, 1,208 patients in six hospitals have been followed every 6 months. The goals of this cohort study are to 1) study the epidemiology of acute and chronic HCV infection, 2) analyze important HCV risk factors, and 3) investigate the efficacy of antiviral therapy and disease outcome due to HCV infection. This study is the first nationwide HCV cohort study in Korea. The data from this cohort will provide valuable information for modifying optimal treatment guidelines for Korean patients infected with HCV.

VIRUS SEQUENCE DATABASE (VSD)

The Virus Sequence Database (VSD, http://vsd.cdc.go.kr) contains hantavirus, rotaviruses, norovirus, enteroviruses, flavivirus, coronaviruses, papillomavirus, hanipavirus, Ebola virus, Marburg virus and arenavirus data. The VSD provides virus sequence and reference information on strains, hosts, geographical origins, genotypes, sampling years, and publication. If a virus sequence in the VSD has location information, the approximate global distribution and prevalence of the selected virus sequence is visualized in a map. Moreover, a data collection project began in 2010 to supply fresh and accurate data to researchers, and a large amount of sequence information was corrected. Only curated sequence information is released to the VSD. The VSD updated the tools service in 2013,

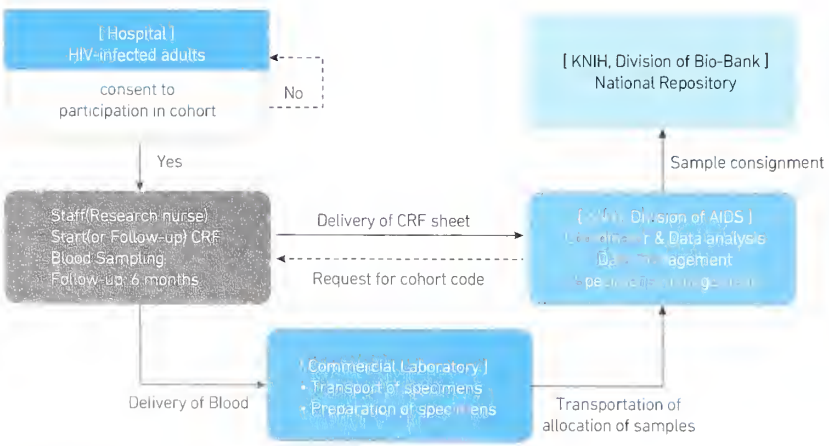


FIGURE 15. Flow of the Korea HIV/AIDS cohort study.

FIGURE 16. Main homepage of Virus Sequence Database (VSD)

which allows researchers to investigate basic and advanced analyses, such as biochemical features and sub-localization of sequences, multiple sequence alignment, BLAST, B-cell epitopes, phylogenetic trees, palmitoylation, and primer design. All data and integrated analyses and visualization in the VSD are currently available as a free service to the virus research community to facilitate research and development of diagnostics, prophylactics, vaccines, and therapeutics against human pathogens and emerging infectious pathogens.

PATHOGEN PROTEOME PROJECT

The appearance of emerging and reemerging infectious diseases remains a major cause of death worldwide (WHO, 2004). Thus, it is necessary to develop a comprehensive strategy to manage infectious diseases. We conducted the Pathogen Proteome Project using immunoproteomic and bioinformatic approaches to identify target proteins for diagnosing and treating pathogens. Antigenic candidates were selected via bioinformatic functional prediction of the genome sequence data from pathogens such as *C. sinensis, Plasmodium vivax, S. pneumoniae,* and *E.coli O157:H7*, and validated the antigenicity. About 270 antigen candidates were cataloged and about 190 (72%) had their antigenicity determined by ELISA, immunoblot, or microarray via expression of recombinant proteins or synthesis of peptides. We tried to determine the epitopes of antigenic proteins using bioinformatic prediction tools. The Pathogen Omics Database (PathOD, http://pathod.cdc.go.kr) was developed to manage pathogenic protein information collected from bioinformatic and immunoproteomic analyses. EpiSys, a pipeline for bioinformatic tools, was developed to predict and identify pathogenic proteins from pathogen genomes. Future research will develop an effective immunoproteomics and bioinformatics analysis system to screen and identify antigens and immunogens as targets for infectious disease control strategies.

ALLERGIC DISEASE RESEARCH PROGRAM

The prevalence of chronic respiratory and allergic diseases including asthma and chronic obstructive pulmonary disease is increasing rapidly due to alterations in the environment caused by climate change. Accordingly, medical expenses are increasing with the spread of chronic disorders in

TABLE 6. The result of antigenicity validation for antigenic candidates from target pathogens

Target Pathogens	Antigen candidates	Determination of antigenicity		
		Recombinant proteins	peptides	Total
Clonorchis sinensis	162	28	80	108
Plasmodium vivax	39	21	17	38
Streoptococcus pneumonia	36	23		23
E. coli O157:H7	33	9	16	25
Total	270	81	123	194(72%)

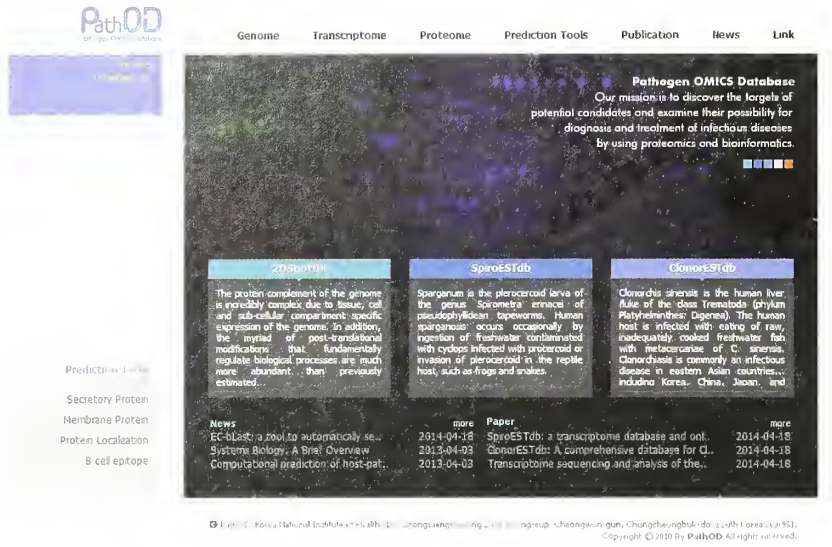


FIGURE 17. The Pathogen Omics Database (PathOD) main page

developed countries.

STANDARDIZATION OF ALLERGIC DISEASES

Although allergic diseases are diagnosed through a combination of examinations such as medical history and clinical testing, there is an absence of standardization, making it difficult to diagnose disease. Therefore, there is an urgent need for nationwide disease controlling technology and standardization. The KNIH built the Center for Standardization of Allergic Diseases in 2009, targeting a research basis for overcoming allergic diseases. In 2013, various efforts to discover and validate allergic disease-associated genetic variants were made via consortia and collaborations in the fields of allergic diseases and genomics. A replication study for previously reported asthma-related genetic variants was conducted from the domestic consortia of allergic diseases. Not only genetic variants but also epigenetic markers were discovered for risk assessment of allergic diseases in the Korean population. The reference ranges for various immunological factors were determined, such as Th1/Th2 and total IgE level in high school and adult populations. In addition, research on standardization for diagnosing and treating allergic diseases has created reference values or standardized protocols for various diagnostic tests, such as the pulmonary function test for children and the oral food challenge for adults. Twelve recombinant allergens were also manufactured and standardized to evaluate main allergens in Korea.

KNIH National Reference Laboratory

INTERNATIONAL LABORATORY INSTALLATION AND COLLABORATIVE RESEARCH ON TROPICAL, VECTOR-BORNE, AND NEGLECTED TROPICAL DISEASES

Laboratories for research collaboration in this network project operate in China, Mongolia, TDR/WHO, and Thailand (Mahidol University). Surveys of parasitic diseases (intestinal parasites, *Babesia* spp., toxoplasmosis, and enteropathogenic protozoa) among residents and vectors (mosquitoes, ticks, and rodents) have been carried out annually. Three trainees from China visited for 1 month and received research training, including the operation of research equipment, epidemiological studies and direct microscopy for intestinal parasitic infections at the KNIH.

JAPANESE ENCEPHALITIS REGIONAL REFERENCE LABORATORY FOR WHO/WPRO

The WHO/WPRO Japanese Encephalitis(JE) laboratory network has been maintained since February 2009. The fourth JE hands-on training was held in September 23–27, 2013, in Osong, Korea. We also conducted a proficiency test (JE IgM ELISA) provided by WHO/WPRO. The fifth informal JE laboratory meeting was held in November in Japan to discuss future plans for the JE laboratory network. A total of 169 human specimens from Malaysia and the Philippines were forwarded to KNIH for further JE confirmatory testing.

Accomplishments

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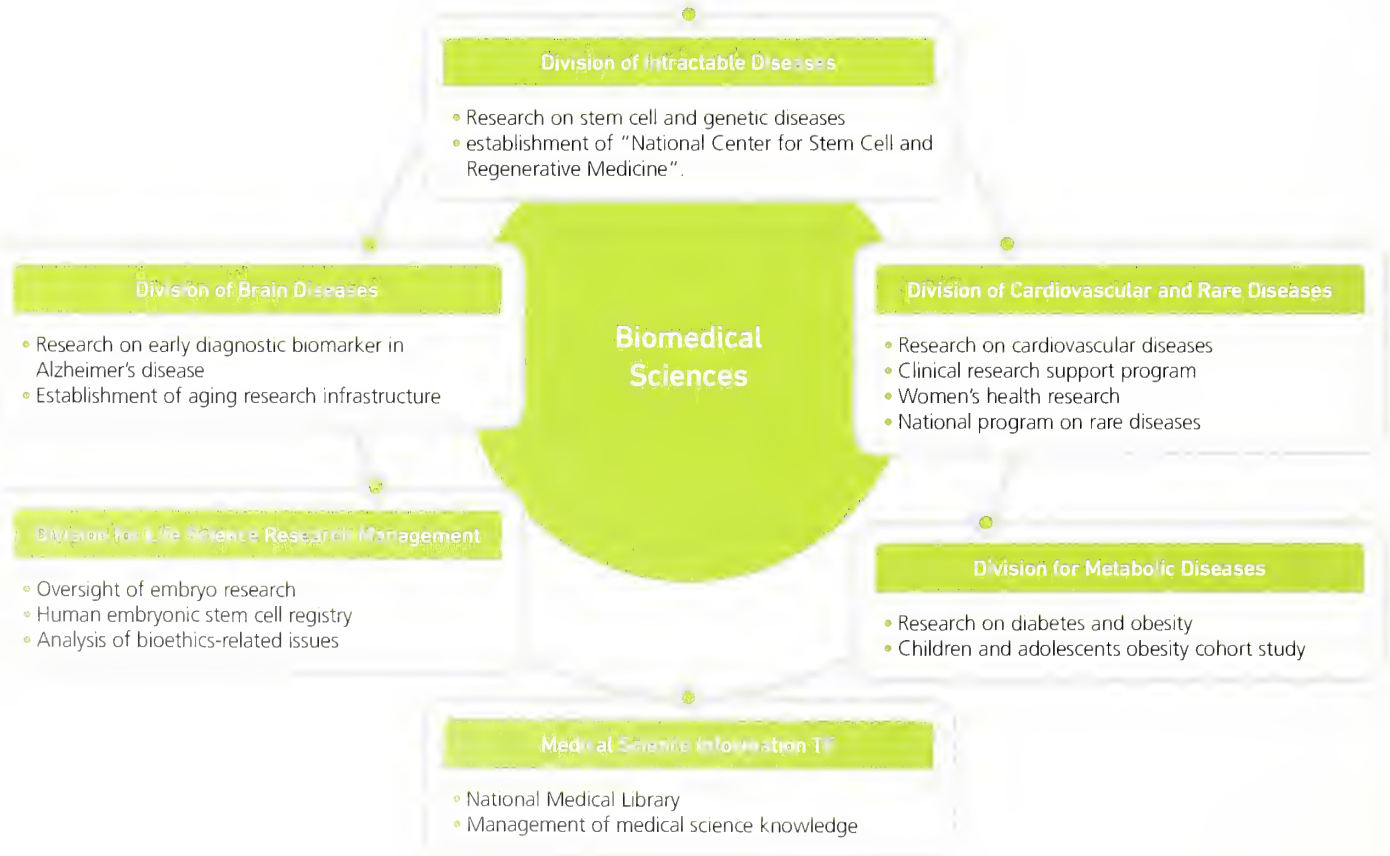
Center for Biomedical Sciences

The goal of the Center for Biomedical Sciences is to improve the quality of human health by developing methods for diagnosis, prevention, and treatment of non-communicable diseases such as diabetes, dementia, atherosclerosis, and genetic diseases. The center encourages evidence-based healthcare by promoting high-quality clinical research and supporting the development of clinical guidelines. It also supports the national program for patients with rare diseases, which includes a subsidy program for medical expenses, an information center, and research programs. Finally, the center supports human embryo research, therapeutic cloning, and genetic tests according to the Bioethics and Safety Act.

The Center's objectives are to:

- Develop effective modalities to prevent and treat chronic diseases
- Foster infrastructure for chronic disease research
- Establish the national clinical research support program
- Support the national program for rare diseases
- Ensure bioethics and safety in biomedical research
- Set up infrastructure for stem cell and regenerative medicine research



Research on Chronic Diseases

NATIONAL RESEARCH INFRASTRUCTURE FOR STEM CELLS AND REGENERATIVE MEDICINE

Regenerative medicine using stem cells has provided innovative developments in the field of therapeutic medicine. Regenerative medicine approaches using stem cells are expected to accelerate as investment and research efforts continue to increase worldwide. Pluripotent stem cells have both infinite proliferative potential and the capacity to differentiate into cell types derived from all three of the embryonic germ layers. The potential of pluripotent stem cells extends beyond their applications in cell-based therapeutics, such as artificial tissues and organs, to modeling human tissues *in vitro* to provide analytical and assessment tools for drug discovery and development.

The National Center for Stem Cell and Regenerative Medicine (NCSR)

We have established the National Center for Stem Cell and Regenerative Medicine (NCSR) as a national research infrastructure for stem cell and regenerative medicine. We would like to act as a bridge between stem cell and regenerative medicine research, and applications, which will accelerate stem cell and regenerative medicine by strengthening infrastructure and standardization. The core values of the NCSR are ethics, standardization, open

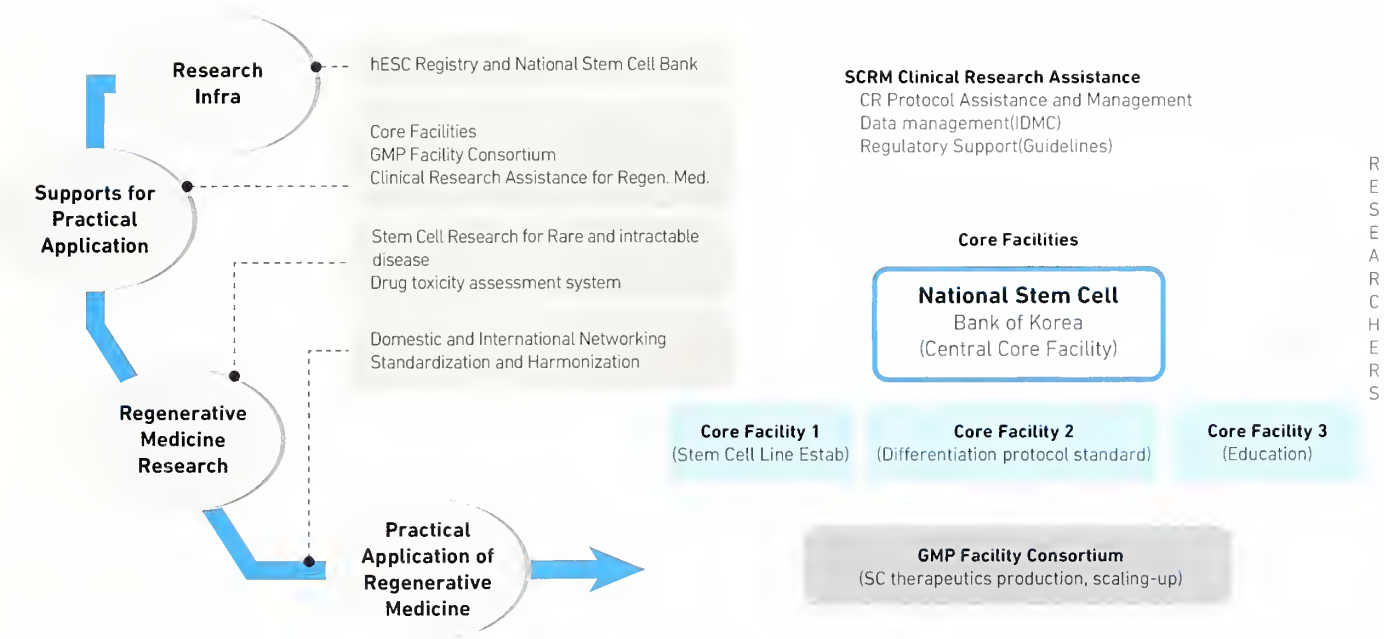


FIGURE 1. Strategies for the National Center for Stem Cells and Regenerative Medicine

sharing, and creativity. The mission of the NCSR is to provide the infrastructure to support and facilitate creative regenerative medicine research by establishing global standards, high-quality stem cell resources and information, and to materialize regenerative medicine while ensuring ethics and safety. The center concentrates on the human embryonic stem cell registry, the National Stem Cell Bank of Korea, data management for stem cells and regenerative medicine, core facilities and the GMP (Good Manufacturing Practices) Consortium, international and domestic networking, and multi-disciplinary research, together in one complex.

In particular, the NCSR was specifically designed to meet the standards of a GMP facility at the international level in terms of the safety and efficacy of medicines. The center committed to a total project cost of 18.9 billion Won, and it will be constructed as a basement and five ground floors (total area 5,181 m^2). The building is expected to be completed in 2015.

Human Embryonic Stem Cell Registry

The Korea Stem Cell Registry (KSCR) was established to increase the credibility of human embryonic stem cell lines established in Korea, and registration became effective on January 1, 2010. The KSCR has characterized the submitted lines with respect to DNA fingerprint, chromosome stability, expression of stem cell markers, and contamination with *Mycoplasma*. The characterization data and ethical aspects, such as informed consent for donation of surplus embryos, were reviewed by a stem cell registry advisory board. A total of 79 domestic and 12 imported human embryonic stem cell (hESC) lines have been registered including six domestic and three imported lines submitted for registration in 2013. Information about the hESC lines is available at the Korea Stem Cell Registry website (htt://kscr.nih.go.kr).

FIGURE 2. A street view of the National Center for Stem Cells and Regenerative Medicine

National Stem Cell Bank of Korea

The National Stem Cell Bank of Korea (NSCB) was launched in 2012 to function as part of the infrastructure for regenerative medicine research. The NSCB focuses on supplying quality-controlled hESCs and pluripotent stem cells (hiPSCs). The NSCB prepared standard operation procedures (SOPs) to establish a standardized banking system for the cell culture process, quality control, and monitoring of hPSCs. In 2013, 33 hPSCs (19 hESCs and 14 hiPSCs) were deposited in the bank. The quality and characterization of two hESCs and three hiPSCs were assessed by SOPs. To distribute the qualified hPSCs, the NSCB is preparing cell banks for pre-master, master, and distribution according to SOPs.

hiPSCs were generated from human dermal fibroblasts or urine cells using three different reprogramming methods such as retrovirus, modified mRNA, and Sendai virus to facilitate research and practical use by scientists. These hiPSCs were characterized and identified to be hESCs in morphology and gene expression profiles. Additionally, primary cells carrying 36 different mutations were collected to make disease-specific hiPSCs, which will provide valuable benefits for researchers.

The International Stem Cell Forum (ISCF) is comprised of 21 stem cell research funding agencies from around the world such as the NIH (USA), the Medical Research Council (UK), and RIKEN (Japan). It was founded in January 2003 to encourage international collaboration and funding support for stem cell research, with the overall aim of promoting global good practices and accelerating progress in this vitally important area of biomedical science. The KNIH has been administered as Korea's representative member institute of the ISCF since 2013. This membership will accelerate participation of the KNIH in the international collaboration for stem cell research by the ISCF.

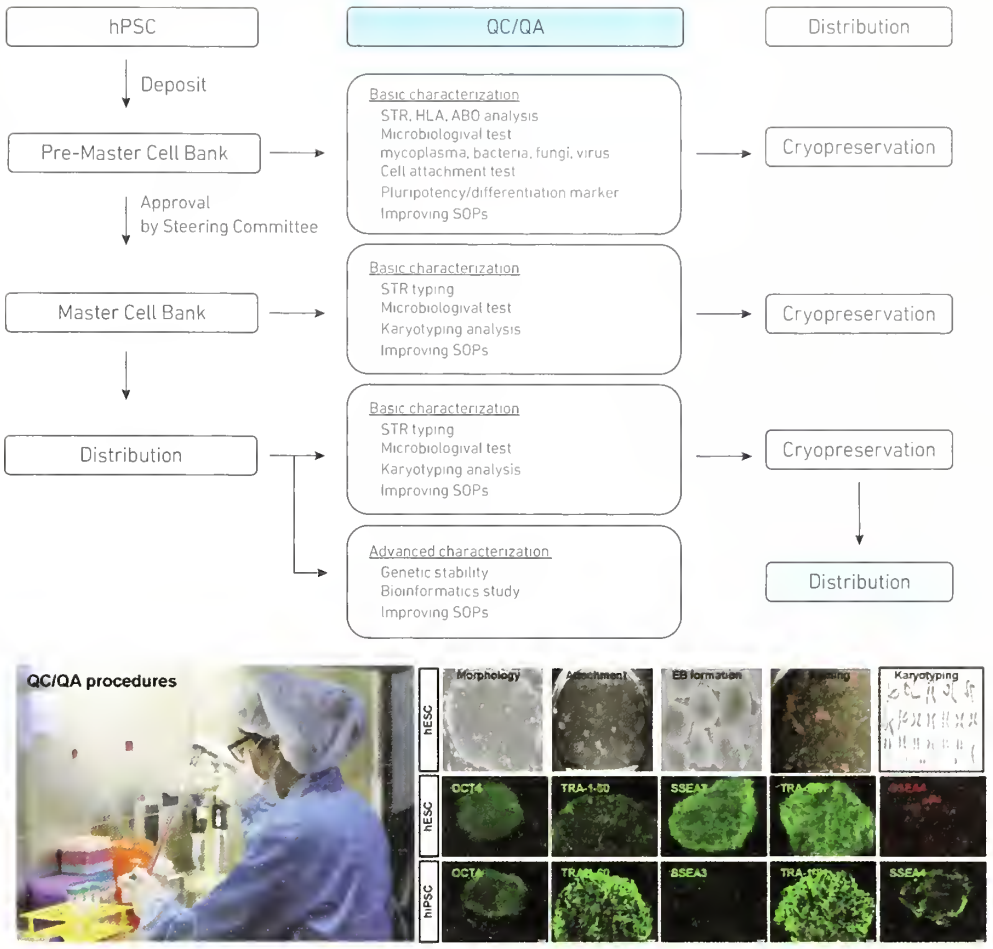


FIGURE 3. Introduction of the National Stem Cell Bank of Korea

ALZHEIMER'S DISEASE AND AGING

Aging is natural and gradual biological impairment. Function declines with age and is accompanied by age-related chronic diseases. We have conducted studies on the mechanisms of aging and the pathogenesis of age-related chronic diseases.

The aging process is the greatest known risk factor for Alzheimer's disease (AD), which is a common form of dementia among older adults. Moreover, AD is an irreversible, progressive brain disease that slowly destroys memory and learning and is regarded as the most common cause of dementia in aging. The two main pathological hallmarks of AD are amyloid plaques and neurofibrillary tangles in the brain. Much research has focused on methods to decrease the amount of amyloid β (Aβ) in the brain to prevent or delay the onset of AD. Our goal was to develop new tools for early diagnosis, treatment, and prevention of AD, which may contribute to healthy aging in humans.

The process of aging is at least partly due to accumulated damage done by reactive oxygen species (ROS). ROS are constantly generated under normal conditions as a consequence of aerobic metabolism. The most common ROS types are superoxide anions (O_2^-), hydrogen peroxide (H_2O_2), and hydroxyl radicals (OH^-). These reactive species are highly toxic to cells and damage a variety of macromolecules, including nucleic acids, proteins, and lipids. Many studies have shown that Aβ may induce the generation of ROS and provide a linkage between oxidative stress and AD pathogenesis.

Cadmium (Cd) increases intracellular ROS levels.

Cd is a highly ubiquitous heavy metal that damages human organs including kidney, liver, and the blood system. Growing evidence indicates that Cd enters the central nervous system(CNS) by changing the permeability of the blood-brain barrier (BBB) and may be associated with neurodegenerative diseases. We have shown that the generation of ROS by Cd activates neuronal cell death through GADD153, which is a member of the C/EBP family of bZIP transcription factors (Kim et al. BMC Cell Biology 2013, 14;4).

In addition, we demonstrated that Cd induces cleavage of N-cadherin via γ-secretase activation in C6 glioma cells (Jo and Koh. Toxicology Letters 2013, 222; 117–121). γ-secretase, which initiates the generation of Aβ is responsible for cleavage of various proteins, including Alzheimer β-amyloid precursor protein (APP), Notch, E-cadherin, and N-cadherin. We have also presented molecular evidence that γ-secretase-dependent N-cadherin cleavage occurs downstream of ROS or Ca^{2+}-mediated ERK activation. Thus, these results suggest that the ERK signaling pathway plays an important role in the processing of N-cadherin, providing a therapeutic target for preventing metastasis of glioma (Jo and Koh. Toxicology Letters 2013, 222; 117–121).

The accumulation of disease-related protein is a characteristic event observed in the pathogenesis of neurodegenerative diseases. Specifically, BACE-1, which initiates generation of Aβ, increases in the brains of patients with Alzheimer's disease. We found that small ubiquitin-like modifier (SUMO)-1 interacts with the dileucine motif of BACE1 and regulates the BACE1 protein level. Our results indicate that SUMO1 is not only a novel and potent regulator of BACE1 accumulation and Aβ generation but is also a potential therapeutic target for Alzheimer's disease (Patent pending; 14/021,210).

We have also discovered a novel function of glutamate carboxypeptidase II (GCPII), that is, degradation of Aβ peptides in the brain. GCPII is a membrane-bound metallopeptidase that catalyzes the hydrolysis of the neurotransmitter N-acetyl-L-aspartyl-L-glutamate (NAAG). The GCPII inhibitor 2-PMPA binds to the GCPII (S1' pocket) glutamate-recognition site. Interestingly, 2-PMPA does not inhibit the Aβ-degrading activity of GCPII, suggesting differential binding of NAAG and Aβ. Using site-directed mutagenesis and an S1 pocket specific inhibitor (compound 2), which was developed by *in silico* computational modeling, we discovered that Aβ degradation occurs through the S1 pocket but not the S1' pocket responsible for NAAG hydrolysis. These results indicate that GCPII has two distinctive binding sites for two different substrates and that Aβ degradation occurs through binding to the GCPII S1 pocket (Lee et al., Biochemical and Biophysical Research Communications 2013, 438;765–771).

We have focused on the discovery of aging-related biomarkers and gene functions using human mesenchymal stem cells (hMSCs) and mouse models related to premature aging, including progeria syndrome. Progeria is caused by a LMNA gene point mutation that generates a truncated form of lamin A, termed progerin. hMSCs are multipotent adult stem cells that show great promise in regenerative medicine. The regenerative potential of hMSCs decreases with aging. Moreover, the age-dependent

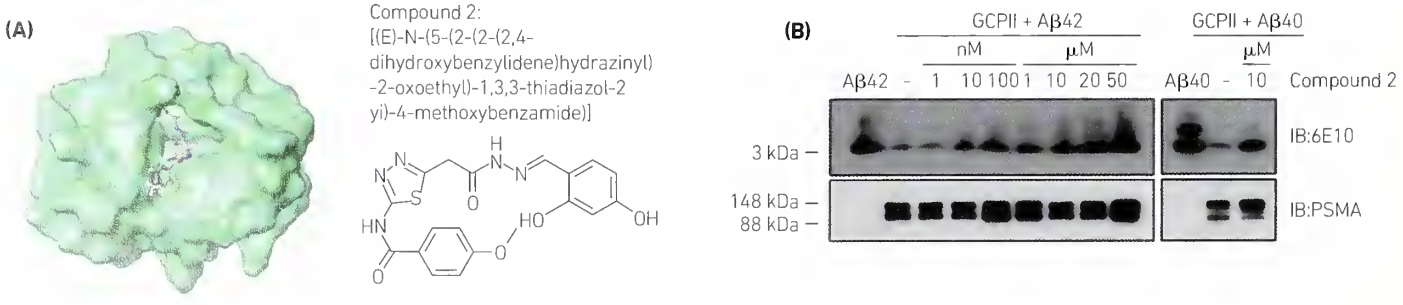


FIGURE 4. Compound 2 specifically inhibits amyloid β (Aβ) degradation. (A) Docking mode of compound 2 to the S1 pocket of glutamate carboxypeptidase II (GCPII) and the molecular structure of compound 2. (B) Purified rhGCPII was incubated with Aβ42 in the presence of compound 2 for 16 h at 37°C and residual Aβ peptides were detected by Western blotting.

decrease in regenerative capacity is linked to a reduction in hMSC cell number with aging. Old hMSCs must be expanded otherwise their use in stem cell therapies will be limited. These obstacles prompted us to investigate a strategy to restore the potential capacity of young cells from old hMSCs to improve the efficiency of stem cell therapy. We investigated candidate aging factors during normal aging and progerin-induced aging at the cellular level. Moreover, we have set up several databases on aging and eight age-related chronic diseases using a text-mining tool (refer to http://www.cdc.go.kr). This tool will provide an interesting and creative research area of overlapping pathways, which are common to the normal aging process and specific chronic diseases, providing us with insights into the regulation of both aging and aging-related diseases by controlling the aging process.

STUDY OF CARDIOVASCULAR DISEASES

The incidence and subsequent social and financial burden of cardiovascular diseases are expected to worsen rapidly as life expectancy increases and lifestyles change. The identification and characterization of molecular pathways leading to atherosclerosis have highlighted several potential therapeutic targets. The aim of this research was to understand the mechanisms by which cardiovascular diseases develop and to provide tools for their diagnosis and therapy. We studied the cell biology of endothelial, smooth muscle and cardiac fibroblast cells, focusing on the mechanisms of autophagy in calcification, a critical early event in atherogenesis, and the antiangiogenic roles of the coronin family. We have established a zebrafish facility to develop human disease models. Zebrafish is a powerful biomedical research tool.

Role of coronin 1B in vascular endothelial growth factor (VEGF)-induced endothelial cell migration

Angiogenesis is new blood vessel formation from pre-existing vessels and is an important step in the development of cardiovascular disease. Endothelial cell migration is a key step during angiogenesis that is regulated by various factors and their downstream signal transduction pathways. Coronins are a family of tryptophan (W) and aspartic acid (D)-repeat actin-binding proteins that regulate various cellular processes, including apoptosis, phagosome formation, and migration. Coronins interact with the actin-related protein 2/3 (Arp2/3) complex and regulate cell motility. In particular, the interaction of coronin 1B with the Arp2/3 complex is regulated by coronin 1B phosphorylation at serine 2, which is mediated by protein kinase C (PKC). Recent studies show that coronin 1B plays a role in vascular cell migration. Williams et al. (Circulation Research 2012) showed that coronin 1B is expressed in vascular smooth muscle cells and its serine 2 residue is phosphorylated by platelet-derived growth factor (PDGF)-activated PKCε. Then, the interaction of coronin 1B with Arp2/3 is decreased leading to migration of vascular smooth muscle cells, suggesting a negative role for coronin 1B in PDGF-induced vascular smooth muscle cell migration. Usatyuk et al. (Plos One 2013) showed that coronin 1B is required for sphingosine-1-phosphate (S1P)-induced endothelial cell migration, suggesting a positive role for coronin 1B in S1P-induced endothelial cell migration. In that study, the authors mentioned that S1P induces phosphorylation of the coronin 1B tyrosine residue rather than that of the serine residue, suggesting that phosphorylation of a different reside (tyrosine vs. serine) may result in an opposite function of coronin 1B during cell migration.

VEGF is a potent angiogenic factor that induces endothelial cell migration. However, the role of coronin 1B in VEGF-induced endothelial cell migration has not been investigated. Thus, we investigated the function of coronin 1B in VEGF-induced endothelial cell migration, focusing on coronin 1B serine 2 reside phosphorylation and Arp2/3 complex interaction-mediated cell migration.

Figure 5 shows that VEGF efficiently induces phosphorylation of the coronin 1B serine 2 residue in a time-dependent manner, suggesting the involvement of coronin 1B in VEGF-induced signal transduction in endothelial cells. Because Usatyuk et al. showed coronin 1B tyrosine residue phosphorylation in response to S1P, we will verify phosphorylation of the coronin 1B tyrosine residue in response to VEGF. We optimized a method to

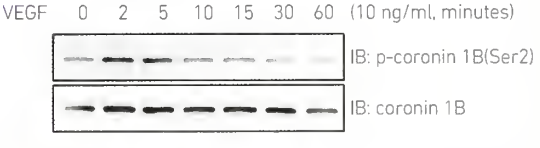


FIGURE 5. Vascular endothelial growth factor (VEGF) phosphorylates coronin 1B at serine2

deplete coronin 1B using siRNA transfection and are preparing a coronin 1B wild-type and mutant over-expression system. Using both depletion and the coronin 1B over-expression system, we will determine the role of coronin 1B in VEGF-induced endothelial cell migration and investigate the coronin 1B-mediated signaling cascade during cell migration, focusing particularly on the interaction with the Arp2/3 complex.

Atherosclerosis and Calcification

Vascular calcification, or rigid deposits of calcium in arterial walls, impairs vasomotion and increases rupture risk in patients with cardiovascular disease (Kelly-Arnold A et al, PNAS USA 2013;110:10741–10746). Thus, vascular calcification promotes significant adverse clinical effects, including systolic hypertension, left ventricular hypertrophy, coronary ischemia, congestive heart failure, and possibly plaque rupture, thrombosis, and myocardial infarction.

Vascular calcification has been classified into three main types of medial Monckeberg arterial, intimal, and infantile calcification. Intimal calcification is mainly associated with atherosclerosis. However, the vascular calcification regulatory mechanism is poorly understood. The main reason of poor understanding is the lack of good animal models for human vascular calcification. Dai *et al.* reported that matrix vesicle release is critical to the process of hyperphosphatemic vascular calcification and that the action is regulated by autophagy. Therefore, we have focused on a mechanistic understanding of vascular calcification and the possibility of preventing atherosclerosis by regulating autophagy. In the present study, we found that an autophagy inhibitor reduced proliferation of vascular smooth muscle cells *in vitro* and *in vivo*. The autophagy inhibitor had an anti-inflammatory effect by reducing VCAM expression. We will also study new therapeutic targets to regress vascular calcification

Research infrastructure for zebrafish disease models

Zebrafish are a popular model system for studying developmental processes and differentiation and have emerged as core experimental systems for modeling human diseases. Zebrafish share similarities in genomic information, organ systems, and organ function with human. Thanks to the rapidly advancing genome editing technology of today, the ease of creating mutant or transgenic lines has facilitated the development of disease models. The field of studying human diseases with zebrafish includes, but is not limited to, tumor diseases, cardiovascular diseases, neuronal diseases, skin diseases, and metabolic diseases.

This project will develop zebrafish disease models to study and cure cardiovascular diseases and has established the Zebrafish Core Facility as part of the national research infrastructure. The opening of the zebrafish facility occurred in September 2013. The zebrafish facility includes aquaria and a stand-alone fish housing unit. Currently, a total of 1,000 fish in 150 aquaria are being kept. More tanks, fish lines, and laboratory space equipped for standard fish work will be established in the near future.

FIGURE 6. Zebrafish housing system

DIABETES AND OBESITY

Type-2 diabetes (T2D) is one of the most prevalent metabolic disorders associated with abnormal lipid and glucose metabolism and it is a principal source of morbidity and mortality worldwide. Its long complications, which can affect almost all parts of the body, include blindness, heart and blood vessel disease, stroke, kidney failure, amputations, and

nerve damage. The prevalence and medical costs for diabetes and obesity care (diabetes, 10.1% prevalence [age, ≥ 30 years, 2010] and $2.25 billion cost (2003); obesity, 31.5% prevalence [age, ≥ 20 years, 2007] and $2.0 billion cost (2005); KNHANES) and the consequent burden on the government are increasing markedly. Therefore, the government must prepare and provide relevant scientific evidence and standard management guidelines or protocols to control and manage diabetes and obesity.

To cope with this our team is leading a national effort to reduce the burden of metabolic diseases such as diabetes, obesity, and metabolic syndrome, by broadening knowledge of the biomedical and physiological mechanism(s) of their pathogenesis. To this, we have identified risk factors for diabetes and obesity and explored how these risk factors are regulated, and determined which specific signaling pathways are associated with the induction of diabetes and obesity. We have developed gene delivery or silencing systems for treatment, which can be directly applied to animal models with metabolic diseases. We used epidemiological analyses and animal models (diseases and transgenic animals), and transformed or primary cell culture-based systems in these studies.

In the epidemiological analyses, we identified specific biomarkers for "early" detection of obesity and related metabolic diseases by targeted metabolite profiling. As childhood obesity is strongly related to future insulin resistance and metabolic syndrome, identifying early biomarkers of obesity-related diseases is useful for controlling future metabolic disorders.

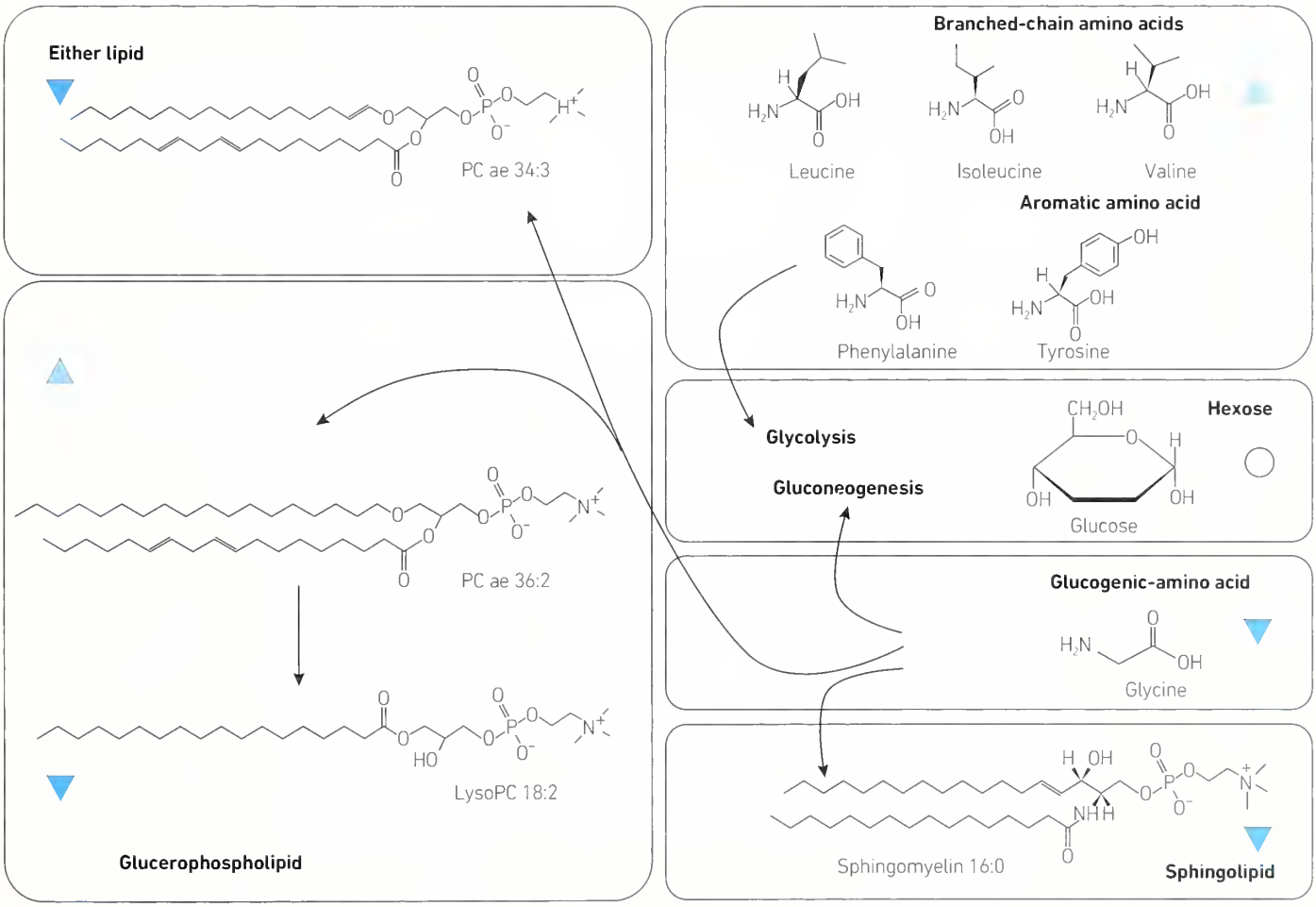


FIGURE 7. Schematic diagram of the metabolites associated with childhood obesity: aa,diacyl; ae, acyl-alkyl; ▲: Increased with obesity, ▼: Decreased with obesity, ○: No change. Arrows represent the direction of reactions.

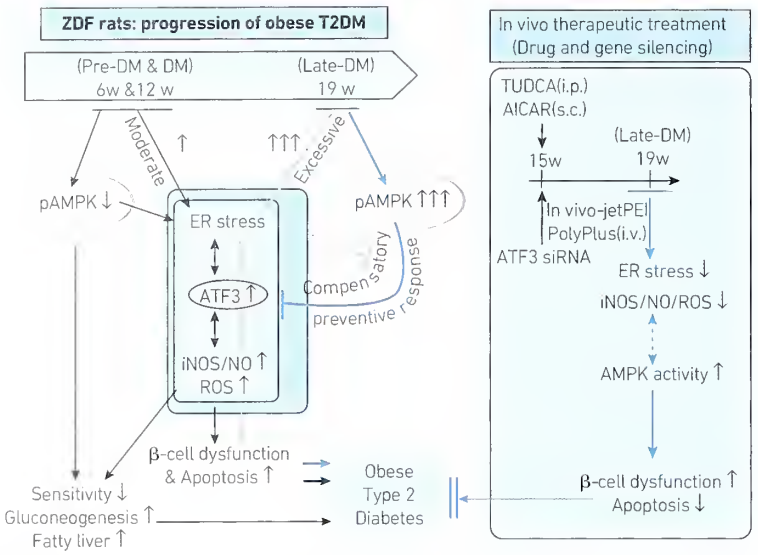


FIGURE 8. The proposed models of in vivo therapeutic treatment in obese type 2 DM

In total, 186 plasma metabolites were analyzed at baseline and after 2 years in 109 Korean boys (age 10.5 ± 0.4 years) from the Korean Children-Adolescents Cohort Study (KoCAS). As a result, we observed that baseline levels of 41 metabolites and 40 metabolites at follow-up were significantly altered in obese children ($P < 0.05$). Specifically, obese children showed significantly higher levels of branched-chain amino acids (BCAAs) and several acylcarnitines and lower levels of acyl-alkyl phosphatidylcholines (PC). Additionally, baseline BCAAs were positively correlated with the homeostasis model assessment for insulin resistance (HOMA-IR) and metabolic risk score at the 2-year follow-up. In logistic regression analyses with adjustments for the degree of obesity at baseline, baseline BCAA concentration (higher than the median value) was identified as a predictor of future insulin resistance risk (HOMA-IR > 3.11; odds ratio, 3.139; $P = 0.042$) and metabolic syndrome (metabolic risk score > 2.07; odds ratio, 3.222; $P = 0.066$). Taken together, BCAAs could be "early" biomarkers for predicting future metabolic diseases. This result appears to reflect the role of BCAAs in modulating insulin secretion, which is related to insulin resistance.

More specifically, BCAAs have glucose metabolism-regulating functions like insulin, including stimulating glucose uptake into cells and inhibiting gluconeogenesis. In the present study, we also observed significant positive associations of both individual and total BCAA concentrations with insulin and glycogenic amino acids (alanine, glycine, and serine) at baseline and follow-up, even after controlling for age and body mass index (BMI) z-scores and waist circumference at each time point. In summary, the ability of BCAAs to predict future risk of metabolic disorders suggests that, like insulin, they can contribute to glucose metabolism. In addition, we observed that the order of BCAAs in the subjects (z-scores) at baseline were well retained after 2 years (after adjusting for BMI and waist circumference; $r = 0.550$, $P < 0.001$ for isoleucine; $r = 537$, $P < 0.001$ for leucine; and $r = 0.362$, $P < 0.001$ for valine). This result indicates that metabolic profiling can be disturbed by obesity even before the age of 10 years, leading to future metabolic pathogenesis including diabetes.

Recently, we have also focused on defining the effects of life-style and/or environmental factors, such as high fat diet (HFD) intake and alcohol consumption, on the progression of type 2 diabetes and obesity. There is evidence that light-to-moderate alcohol consumption may have some beneficial effects in particular regarding cardiovascular events, insulin resistance, and T2D through augmenting glucose-stimulated insulin secretion (GSIS) and insulin sensitivity. None the less it is generally believed that the administration of moderate amounts of ethanol to rodents with metabolic disorders may increase the risk of complications in metabolic diseases or diabetes. Our previous work showed that chronic alcohol consumption could trigger the development of type 2 diabetes by down-regulating the activity of glucokinase (GCK) via tyrosine nitration. Furthermore, we are further studying to identify a novel signaling pathways or regulatory factors that are associated with pancreatic β-cell dysfunction and apoptosis induced by chronic alcohol consumption. At present, we know that the expression of activating transcription factor 3 (ATF3), a stress-inducible transcription factor, and its binding to the putative ATF/CREB site on GCK promoter are increased. And also, in vitro ethanol-induced ATF3 inhibited the positive effect of PDX-1 on GCK transcriptional regulation, enhanced recruitment of HDAC1/2 and histone H3 deacetylation, and subsequently augmented the interaction of HDAC1/PDX-1 on the GCK promoter, which were diminished by ATF3 siRNA. On the other hand, it was also well established that obesity is a

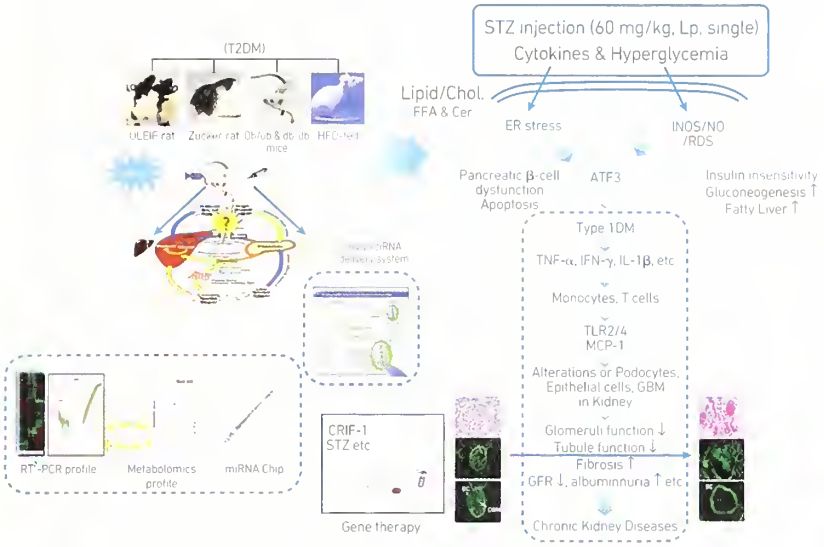


FIGURE 9. Models used to study diabetic kidney failure

major underlying pathology in association with the development of type 2 diabetes, which occurs as pancreatic β-cells fail to compensate for increased insulin demand when the body becomes insulin resistance and hyperglycemia. However, the exact mechanisms and target regulatory molecules by which ER stress, which is highly induced in obesity, drives type 2 diabetes, remain uncertain. Therefore, by using an obese Zucker diabetic fatty (ZDF) rats, a commonly used T2D animal models, we could demonstrate the role of ATF3 on the preventive regulation of AMPK against ER stress-mediated β-cell dysfunction during the end-stage progression of hyperglycemia in ZDF rats. The impaired glucose metabolism and β-cell dysfunction were significantly increased in late-diabetic phase 19-week-old ZDF rats. Although the reduced phosphorylation level of AMPK in 6- and 12-week-old ZDF rats was remarkably increased at 19 weeks, the increases of lipogenice genes, ATF3, and ER stress or ROS-mediated β-cell dysfunction were still remained, which were attenuated by in vivo-injection of chemical chaperon tauroursodeoxycholate (TUDCA), chronic AICAR, or antioxidants. ATF3 did not directly affect AMPK phosphorylation, but counteracts the preventive effects of AMPK for high glucose-induced β-cell dysfunction. Moreover, knockdown of ATF3 by delivery of in vivo-jetPEI ATF3 siRNA attenuated ER stress-mediated β-cell dysfunction and enhanced the beneficial effect of AICAR. Our data suggest that ATF3 may play as a counteracting regulator of AMPK and thus promote β-cell dysfunction and the development of type-2 diabetes suggesting a potential therapeutic target in treating type-2 diabetes (Figure 8).

One of the most promising areas in diabetes research is to identify biomarkers associated with the development of diabetes or diabetic complication diseases including kidney nephropathy, cardiovascular diseases, renal diseases, etc. Among them, we were interested in identifyig major regulatory mechanisms and the preventive tools for diabetic kidney failure since diabetes is considered as the most common causing factor of kidney failure, which accounts for nearly 45.2 % (Korean society of nephrology, 2011). It was well known that diabetic nephropathy, an end-stage disease of diabetic complication, is characterized by fibrosis of renal glomerulus and tubulointerstitial region, but the exact molecular mechanisms by which diabetes may initiate or exacerbate kidney failure remain elusive. To this, we have established the diabetic kidney injury animal models by injecting with streptozotocine (STZ), a potent drug for inducing type 1 diabetes. Here, we observed that STZ-injected rats exhibited renal dysfunction, as evidenced by increased glomerulus, thickened basement membrane, and increased mesenterium, which are correlated with the marked accumulation of ECM molecules and ATF3. In addition, we found high lactate levels in STZ-injected rat urine by metabolomic analysis. We are working to determine whether aerobic glycolysis and lactate are involved in the development of this disease. In the future, we will perform functional studies based on epidemiological data from a children's cohort, and further develop cohort of clinical patients with diabetes and associated risk factors of interest, such as alcohol consumption, to come up with protective and therapeutic strategies for diabetes and obesity (Figure 9).

Epidemiological Studies of Chronic Diseases

REGISTRIES AND COHORTS TO STUDY CARDIOVASCULAR DISEASES

Well planned, designed, and conducted registries and cohort studies provide useful clinical information, patient outcomes, safety, and associations with potential risk factors. Large-scale community-based cohort studies allow us to identify more general causal factors between the incidence of diseases and exposure. Patient registries can be a powerful tool to observe the course and natural history of disease; to understand the relationships between treatments and patient outcomes, the various risk factors that influence disease prognosis, and to assess the safety and effectiveness of various treatment strategies.

Geriatric Disease Prevention and Management Cohort: The KURE (Korea urban and rural elderly cohort) cohort

Population aging has become a global trend, particularly in South Korea, where Korea is expected to enter a super-aged society at an unprecedented speed. Thus, geriatric diseases will begin to cause a big burden on public health and society. Therefore, we will study the epidemiological characteristics of the major severe disorders of the elderly by establishing and managing a Geriatric Diseases Prevention and Management Cohort. In addition, we plan to build a community-based cohort and follow-up more than 5,000 elderly Koreans over 10 years to create disease prevention and management indices.

This research involves: 1) recruiting 1,000 elderly citizens and registering them into the cohort 2) collecting clinical examination data and baseline specimens from participants, 3) building a management system to utilize data and resources, 4) acknowledging the basic characteristics and prevalence of major diseases beginning the first year of the cohort. We selected two communities within Korea and recruited and examined subjects aged > 65 years.

We recruited 2,025 study subjects from 2012 to 2013 through several methods: establishing a community base, conducting a random population survey (recruiting people from the street), and promoting volunteer participation through a poster-based advertising campaign. In 2013, we recruited 1,098 elderly people > 65 years, and conducted health examinations and surveys and collected blood samples. A total of 751 females accounted for 68.40% of all participants I. The average age was 72.01 years, and the major group, which was 39.25% of the total, was 70–74 years.

Registry (prospective cohort) for heart failure in Korea

The prevalence of heart failure has been increasing rapidly in Korea and its influence on mortality, morbidity, and the cost of healthcare is growing rapidly. To reduce the health burden of heart failure and to improve the care system and clinical guidelines, it is essential to verify patient profiles, current trends of management, and outcomes.

The Korean acute heart failure registry (KorAHF) evaluates clinical characteristics, management, hospital course, predictors of mortality, and short- and long-term outcomes of patients hospitalized for acute heart failure syndrome (AHFS) in Korea. The patients hospitalized for AHFS in 10 regionally representative tertiary university hospitals have been consecutively enrolled between March 2011 and July 2013.

This enrollment is supposed to be carried out for 8 years. The study is expected to complete the enrollment of at least 4,500 patients in 2013 and to follow-up until 2016.

This study was conducted in 2013, as shown below. The enrollment and follow-up of the prospective registry, the KorAHF, have begun in earnest this year. As of November 22, 2013, 5,294 subjects with acute heart failure have been enrolled in the registry, and we estimate to enroll 4,500 subjects this year. Data management and audits have been performed for quality control of the registry. The case report form has been upgraded based on the results from data management and the audit. A total of 4,183 patients were used for the interim analysis. This analysis was performed to investigate baseline characteristics, management, short-term outcomes, and predictors of in-hospital mortality.

Mean age was 69 ± 14 years; 54% were male; 51% had *de novo* HF; 59% had hypertension; and 36% had diabetes mellitus. Ischemia was both

TABLE 1. Characteristics of patients enrolled in the Korean acute heart failure registry (KorAHF)

	KorAHF	ATTEND	ADHERE	OPTIMIZE-HF	EHFSII
Country	Korea	Japan	USA	USA	Europe
Time period	2011.3~	2007.5-(2012.9)	2001.9-2004.1	2003.3-2004.12	2004.10-2005.8
Sample size	4183/4500	1110 (2009.6)	105388	48612/5791(330)	3580/2981
Follow-up duration	>3 years	180 days	N/A	60,90 days	3-, 12-month
Demographics					
Age	69 (14)	73 (14)	72 (14)	73 (14)	70 (13)
Male	54%	59	48	48	61
Comorbidities					
HTN	59%	71	75	71	63
DM	36%	34	44	42	33
Stroke	15%	12	17	16	13
AF	27%	40	31	31	39
Chronic lung disease	11%	9	31	28	19
Etiology					
Ischemic	38%	33	57	46	30
Clinical status on admission					
De novo HF	51%	63	24	13	37
Creatinine	1.5 (1.5)	1.4 (1.5)	1.8 (1.6)	1.8 (1.8)	N/A
Heart rate	91 (26)	99 (30)	N/A	87 (22)	median 95
SBP	136 (30)	147 (38)	144 (33)	143 (33)	median 135
LVEF <40%	54%	57	47	48.8	46
Management					
IV diuretics	74%	80	87	N/A	84
IV inotropes	32%	21	8	7	<29.8
IV vasodilators	41%	less than 46	7	14	38
ACE inhibitors/ARBs	66%	N/A	67	N/A	80
Aldosterone antagonist	44%	N/A	20	N/A	48
beta-blockers	49%	N/A	74	N/A	61
Outcome					
length of stay (median)	9 days	21	4.3	6.4 (mean)	9
in-hospital mortality	**6.1%(including TPL)**	7.7	3.8	3.8	6.7

the leading cause (38%) and the most frequent aggravating factor (24.3%). Parenteral diuretics and ionotropics were administered in 74% and 32% of patients, respectively. Angiotensin converting enzyme inhibitors/angiotensin receptor blockers and beta-blockers were prescribed at discharge to 66% and 49% of the patients, respectively. The mean length of hospital stay was 9 days and the mean cost for admission was about 9,164,200 Korean Won. In-hospital mortality was 6.14% (including 1.38% of patients who underwent urgent heart transplantation). After discharge, 90-day mortality was 4.2% and the rehospitalization rate was 15%(Table 1).

The prevalence of diabetic kidney disease is increasing rapidly in Korea according to a report by the Korean Society of Nephrology (KSN). About 30~40% of type 2 diabetes mellitus cases develop to diabetic nephropathy, with an associated increase in morbidity. Diabetes is the most common causal factor of kidney failure and accounts for nearly 45.2% of cases (KSN, 2011). We believe that if diabetes is well controlled, the prevalence of chronic kidney disease and kidney failure will decrease remarkably. Thus, we are also interested in identifying major regulatory mechanisms and the preventive tools for diabetic kidney failure. Our studies have focused on the following: 1) establishing animal models of diabetic kidney failure; 2) identifying the roles of cytokines in the development of kidney (tubular and mesangium) fibrosis; 3) identifying new target regulatory genes using gene profiling, metabolomics, and target gene chip analyses; 4) conducting an *in vivo* intervention study (using a gene-delivery system or drugs) to control the development of kidney failure; and 5) identifying early diagnostic biomarkers based on scientific evidence.

By studying the characteristics and the associated causes of heart failure, we can establish the basis for evidence-based disease management. Moreover, we can improve prognosis via active management of heart failure and collect basic data for economic feasibility. In addition, through active management of heart failure, the economic feasibility of improving the prognosis can be deduced from the underlying data. All of these activities could be very helpful for establishing public health policies in the future.

Prospective Cohort Study for the Acute Myocardial Infarction Prognostic and Surveillance Index

Cardiovascular disease is one of the four leading causes of death in Korea. Among cardiovascular diseases, acute myocardial infarction (AMI) is the first cause of death in the U.S., and shows an increasing prevalence in Korea. The prognosis of patients with AMI can be improved by proper treatment and management. Thus, many western countries have adopted AMI registries, in contrast to Korea, which lacks nationwide AMI research sources. Therefore, a long-term prospective, government oriented, nationwide cohort study for AMI is necessary. The aim of the present study was to develop a prospective observational study model for an AMI prognosis and surveillance index for AMI prevention and management.

The present study will 1) analyze the current status of a prospective AMI registry in Korea and other countries, 2) develop a prospective observational study model for an AMI prognosis and surveillance index based on an analysis of the risk factors, economic burdens, major complications, and prognostic factors in patients with AMI, 3) develop AMI management plans, and 4) apply public health policies. We constructed a nationwide AMI registry network, and suggest a fundamental research model for developing a prospective observational study model for an AMI prognosis and surveillance index.

Studies show that from March 2013 to October 2013, 3,122 patients were properly diagnosed with AMI [mean age, 64.6 ± 12.4 years, 2,344 men (75.1%)]. Of these, there were 1,482 (47.5%) ST-segment elevation myocardial infarction cases, and 1,640 (52.5%) non ST-segment elevation myocardial infarction cases. A total of 58.8% had a smoking history. Hypertension was detected in 51.2%, diabetes mellitus in 29.2%, hyperlipidemia in 11.1%, and a family history in 6.8% of all patients. In total, 2,436 (78.0%) patients complained of typical chest pain and 789 (25.3%) of dyspnea. Mean systolic blood pressure at the time of admission was 128.2 ± 29.1 mmHg, diastolic blood pressure was 77.4 ± 17.0 mmHg, and mean heart rate was 78.6 ± 19.6 beats/min.

A coronary angiogram was performed in 3,066 (98.2%) and percutaneous coronary intervention (PCI) was performed in 2,694 (86.3%). Thirty-seven (1.4%) patients underwent coronary artery bypass graft. A total of 1,461 patients (46.8%) had one-

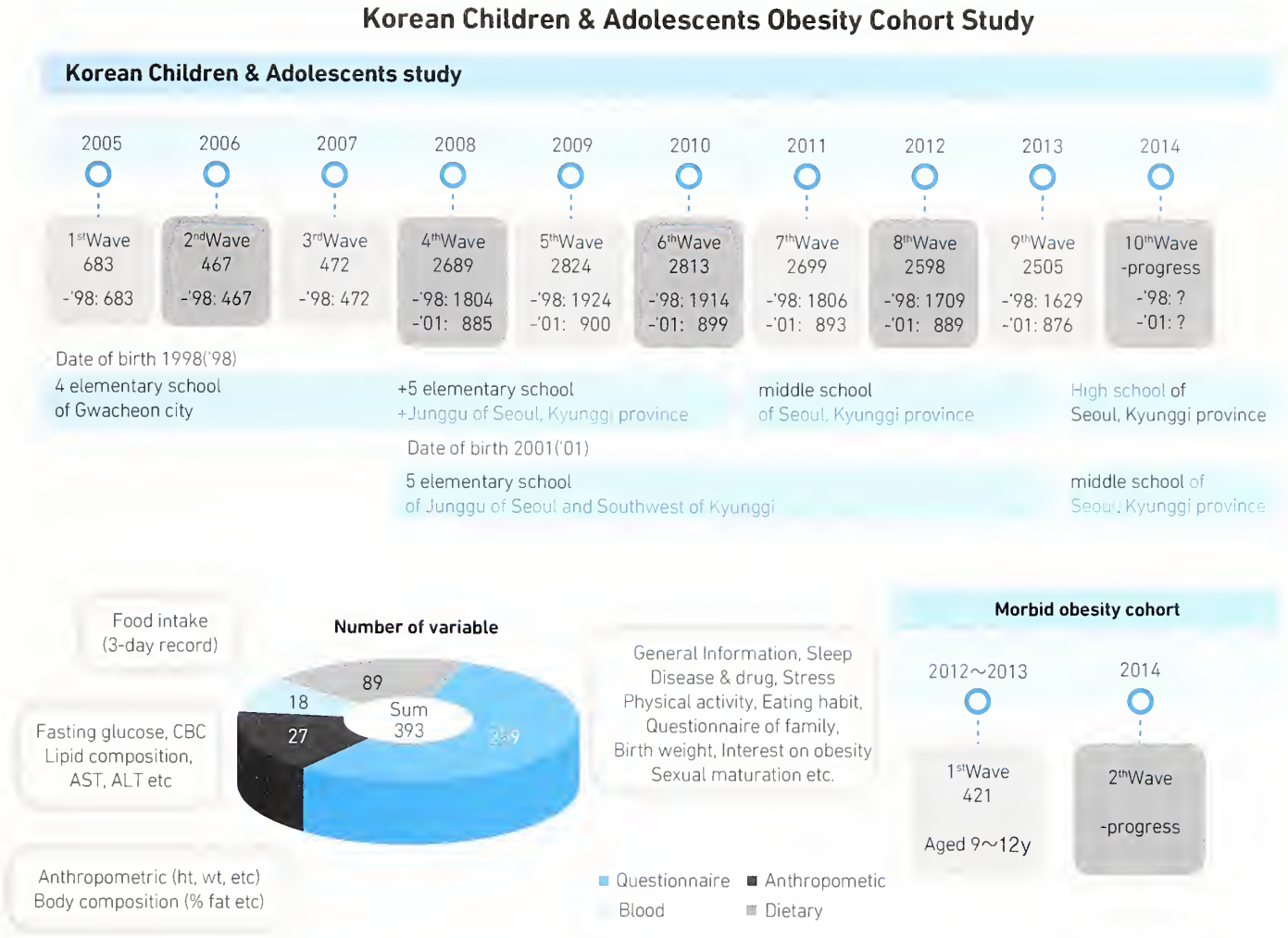


FIGURE 9. Follow-up flow chart of the Korean children and adolescent obesity cohort study

vessel disease and 859 patients (27.5%) had two-vessel disease. Three-vessel disease was observed in 487 patients (15.6%). Complex arterial disease with a left main stem lesion was displayed in 315 patients (10.1%). The infarct-related artery was the left anterior descending artery in 1,461 patients (46.8%), the left circumflex artery in 524 (16.8%), the right coronary artery in 1,058 patients (33.9%), and the left main stem in 78 patients (2.5%). PCI was successful in 2,648 of 2,694 patients. In-hospital mortality was 4.2% (132 patients) in all patients and 3.6% (112 patients) in patients who underwent PCI.

These results will be used as baseline data to develop strategies and policies for preventing and managing AMI. These results will provide baseline data and a model for future AMI studies.

Korean Children and Adolescent Obesity Cohort Study

Concurrent with the remarkable increase in adult obesity, Korean children have also become heavier. A proportion of children (age, 6–11 years) exceed the 95th percentile cut-off for the 2007 KCDC BMI on the age growth chart, and children with a BMI > 25 kg/m^2 increased from 5.8% in 1998 to 8.8% in 2010 (1998, 2010 KNHNES). Childhood obesity typically persists into adulthood and increases the risk for insulin resistance and metabolic syndrome-related conditions, such as increased blood pressure

and abnormal glucose and lipid metabolism. Therefore, early detection of individuals at risk of being overweight may have long-term health benefits.

The objectives of the children and adolescent obesity cohort study were to prospectively investigate children's health and lifestyles and to collect biological samples. By analyzing the data collected from this cohort, such as changes in many indices purported to be related to obesity and metabolic disorders in children, we may be able to identify biomarkers for childhood obesity and to assess the efficacy of preventive or therapeutic measures.

The subjects in this cohort were followed from their enrollment in elementary school at age 7 years until the third grade of middle school, when students were aged 16 years. The children were recruited from the city of Gwacheon. in 2005. Anthropometric and genetic data, such as common disease gene variants, metabolic disease indices, and information on food and nutrient intake were collected from the children. Another 1,900 subjects (age, 7 and 10 years) who were living in Junggu, Seoul, and the southwestern area of Kyunggi province, were added in 2008. We are following the subjects recruited from the city of Gwacheon, Junggu, Seoul, and the southwestern area of Kyunggi Provinces as they enter middle school in 2011, and are enrolling new participants from the middle schools of the follow-up subjects. The morbid obese adolescent cohort study was newly conducted in between 2012 and 2013 as part of the children and adolescent obesity cohort study in Seoul and Kyunggi provinces, Korea. The objective of this study was to identify predictors of morbid obesity and associated metabolic disease in Korean children. Subjects who exceed the 99th percentile cut-off for the 2007 KCDC BMI on the age growth chart or who had a BMI > 30 kg/m^2 were recruited. Recruitment for the cohort of qualified participants was carried out at Seoul-Paik Hospital, Inje University and Hallym University Medical Center. We will conduct a follow-up survey every year.

We expect this project to generate new findings on obesity candidate genes and variants and hope to develop an obesity prevention program. The previously performed clinical and physiological examinations and lifestyle data collection will be repeated. We will continue to collect information on children's health and to identify biomarkers associated with the development or control of childhood obesity to develop protective and therapeutic approaches.

Women's Health Research

EPIDEMIOLOGICAL STUDIES

The Korea Nurses' Health Study (KNHS) is a prospective cohort study conducted to evaluate the association between lifestyle and Korean women's health. We collaborated with the Korean Nurses Association and Harvard University to perform this study. It was established in 2013, and female registered nurses aged 20–45 years were enrolled in the cohort if they responded to the web-based self-administered questionnaires. The participants provided information regarding lifestyle and health conditions every 6 months during the follow-up period.

Shift work is considered a risk factor for obesity, and several studies show significant correlations between shift work and weight gain, overweight, or obesity. Previous findings suggested that the risk of overweight and/or obesity for shift work may increase by at least 39%. To investigate the association between shift work and BMI among female nurses, 5,149 current shift workers were selected. The prevalence of overweight/obesity and obesity significantly increased with increasing shift work duration. Additionally, participants with the longest duration of shift work were 1.63 (95% CI, 1.22–2.17) times more likely to be overweight or obese than those with the shortest duration (Table 2). This study included a large, representative sample of nurses in Korea and confirmed an association between increasing duration of shift work and overweight/obesity after considering obesity-related lifestyle factors as confounders. Therefore, special attention should be paid to female nurses with a

TABLE 2. Multivariate-adjusted odds ratios (ORs) for overweight/obesity and obesity according to tertile of shift work duration

Variables		Unadjusted			Model 1		
		OR (95% CI)		*P*-value	OR (95% CI)		*P*-value
	Shift work duration						
BMI < 23kg/m vs. BMI ≥ 23 kg/m^2	Tertile 1	1	(reference)		1	(reference)	
	Tertile 2	1.37	(1.09-1.73)**	0.007	1.11	(0.87-1.42)	0.404
	Tertile 3	3.40	(2.77-4.19)***	<0.001	1.63	(1.22-2.17)**	0.001
	Shift work duration						
BMI < 25kg/m^2 vs. BMI ≥ 25 kg/m^2	Tertile 1	1	(reference)		1	(reference)	
	Tertile 2	1.59	(1.12-2.25)*	0.010	1.21	(0.84-1.74)	0.315
	Tertile 3	3.30	(2.41-4.53)***	<0.001	1.37	(0.91-2.09)	0.135

CI, confidence interval; Model 1: adjusted for age, current smoking status, regular drinking habit, skipping breakfast, regular exercise, marital status, family income, education, sleep problems and self-perceived health status; *P < 0.05, **P < 0.01, ***P < 0.001

long duration of shift work.

Menopause is a physiological phase characterized by permanent cessation of the menstrual cycle in women due to loss of ovarian follicular function. During the menopausal transition, women experience various physical, psychological, and social changes that may affect their quality of life. Regular physical activity may be an effective way to prevent or attenuate menopause-related symptoms. To investigate the influence of physical activity on perimenopausal-associated symptoms, 732 perimenopausal women aged 44–56 years, who visited a health-screening center, were selected as participants. Physical activity was significantly associated with total Menopause-specific Quality of Life (MENQOL) score and the psychosocial and physical subscores. Compared to women in the low physical activity group, women with a moderate level of physical activity had a significantly lower MENQOL total score (ß = −0.306, *P* = 0.028)

TABLE 3. Multiple linear regression analysis of the relationship between Menopause-specific Quality of Life (MENQOL) total score/ subscores and physical activity levels

Variables	Total		Vasomotor		Psychosocial		Physical		Sexual	
	ß	*P*-value	ß	*P*-value	ß	*P*-value	ß	*P*-value	ß	*P*-value
Physical activity										
Low	Reference		Reference		Reference		Reference		Reference	
Moderate	-0.306	0.028	-0.054	0.754	-0.414	0.012	-0.446	0.002	-0.309	0.116
High	-0.190	0.271	-0.154	0.473	-0.053	0.795	-0.184	0.304	-0.369	0.129

Beta coefficients and P-values are presented. The regression analysis was adjusted for age, body mass index, marital status, family income, education, and parity.

and psychosocial (ß = −0.414, P = 0.012) and physical symptoms (ß = −0.446, P = 0.002) (Table 3). These results show that a moderate level of physical activity was associated with reduced psychosocial and physical menopausal symptoms in perimenopausal Korean women.

In addition, we investigated the association between sleep duration and obesity, including both abdominal and general obesity, among middle-aged women in Korea. Compared to women who slept > 7 hours per night, women who slept < 6 hours had significantly greater ORs for both abdominal obesity (OR, 3.16; 95% CI, 1.77–5.63) and general obesity (OR, 2.54; 95% CI, 1.45–4.45) in the older, not the younger, women after controlling for potential confounders.

Hypertension is a well-known leading cause of cardiovascular events and is a great burden on vulnerable classes of people in the healthcare system. Our study examined whether there is a gender difference in the association between socioeconomic status, measured by education attainment, and hypertension incidence using the Korean Genome and Epidemiology Study (KoGES) community-based cohort data. Participants were classified into four categories by self-reported education attainment: highest (>13 years); mid-highest (10–12 years); mid-lowest (7–9 years); and lowest (<7 years). According to the education categories, the prevalence rates of hypertension were 36.7%, 36.3%, 44.4%, and 49.9% in men and 18.6%, 21.4%, 32.0%, and 51.5% in women. Compared to the highest education group (referent), the hazard ratios (95% CI) for incident hypertension across education attainment categories were 1.22 (0.74–2.00), 1.93 (1.18–3.15), and 2.32 (1.43–3.77) (P for trend < 0.0001) in women and 1.04 (0.81–1.33), 1.34 (1.03–1.74), and 1.46 (1.10–1.93) (P for trend = 0.0070) in men, after adjusting for conventional risk factors. Educational level had a stronger impact on hypertension in Korean women than in men. Therefore, a stratified intensive approach for women of low socioeconomic status, particularly those with low education attainment, is needed to prevent hypertension.

Despite the risks of premature birth and other neonatal health concerns, the state of neonatal care in Korea lags behind other advanced countries. The Korean Neonatal Network KNN) was established and launched to form an infrastructure for data collection, study national neonatal intensive care, and ultimately improve the quality of high-risk newborn survival by reducing major complications. Fifty-two neonatal intensive care units (NICUs), which care for 70–80% of total very low birth weight infants (VLBWI) born at < 1,500 g annually (about 3,000 cases), have started to participate sequentially in the KNN. The KNN will be an infrastructure for active and productive multi-center research to improve the quality of various NICU clinical trials and develop Korean-style guidelines or strategies for NICU management.

EXPERIMENTAL RESEARCH

Drinking alcohol during pregnancy poses serious health risks to the unborn child such as prematurity, low or high birth weight, fetal death, and fetal alcohol syndrome. However, the effects of maternal ethanol consumption before pregnancy on abnormal development of the fetus and the association with impaired glucose tolerance of the mother are not fully understood. Six-week-old C57BL/6J female mice were fed a 5% ethanol-containing liquid diet for 2 weeks before pregnancy and the effects of ethanol pre-exposure on fetal development were examined during a subsequent pregnancy. We found that pregnancy or fertility rates decreased in ethanol-fed mice and were correlated with delayed eye formation and the formation of defective toes. Additionally, birth weights of postnatal 0 day (P0) ethanol-fed mice were higher than those of pair-fed mice, but growth was retarded in P14 and P21 offspring of ethanol-fed mice. The macrosomia phenomenon in ethanol-fed mice is strongly associated with dysregulation of glucose metabolism and triglyceride accumulation in the maternal liver during pregnancy. Gut-derived serotonin (GDS) and hepatic inflammatory chemokines and cytokines also increased markedly in ethanol-fed mice, followed by alterations of glucose metabolism in the liver and pancreatic b-cells. Alcohol intake changed the expression of GDS-response receptors from Gs-GPCR to Gi or Gq-GPCR. Detrimental fetal development and impaired glucose metabolism by maternal pre-exposure to ethanol were strongly attenuated by injecting the CYP2E1 inhibitor 4-methylprazol. Taken together, our results suggest that consuming ethanol before pregnancy is a major factor for detrimental fetal development due to maternal metabolic disorders, particularly dysregulation of glucose or insulin metabolism in the maternal liver and pancreatic b-cells(Figure 11).

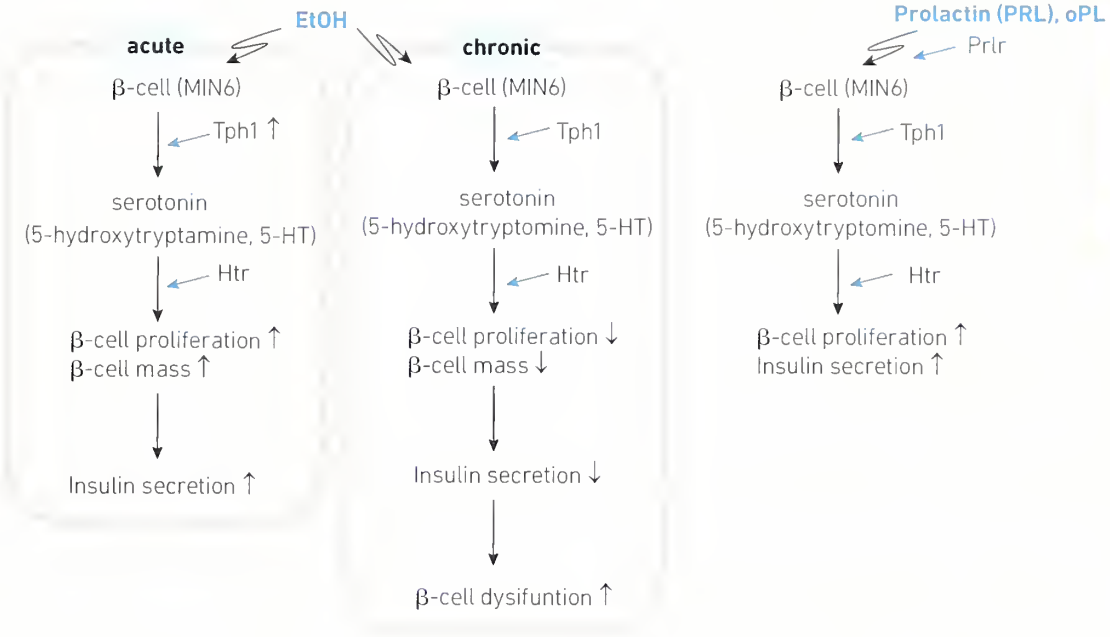


FIGURE 11. Model of Ethanol action on pancreatic beta cells and insulin secretion

National Program on Rare Diseases

Background

"Rare" diseases are those unknown by health professionals, and do not attract interest from the pharmaceutical industry because the potential market is too limited. In Korea, rare diseases are defined based on the KFDA official notice as those affecting < 20,000 patients and for which no appropriate treatment or substitute treatment modality is available. The low prevalence of rare diseases prevents competitive development of therapies in the open market. This results in a lack of information, research, diagnosis, treatment, and expert availability related to rare diseases, ultimately preventing patients from receiving needed health resources and services. A national intervention program including subsidies, information, and research is required.

Objectives

To improve the quality of life of patients with rare diseases as follows:

- Support patients by reducing the medical expense burden
- Provide information about rare diseases
- Help regional patients by reducing indirect expenses

To promote research on rare diseases:

- Construct an infrastructure for rare disease research
- Lead basic research on rare diseases
- Establish clinical research networks.

Major Activities

The national program on rare diseases was launched in 2001 as a subsidy program for medical expenses. Since then, the government has expanded support for patients with rare diseases and promoted a research program.

The subsidy program has some unique characteristics. First, enrolled patients (low-income subjects with any of 138 specified rare diseases) are exempted from the out-of-pocket health expenditures normally associated with coverage under the National Health Insurance plan. The number of patients has increased each year, reaching 24,586 in 2013. Second, caregiver time,

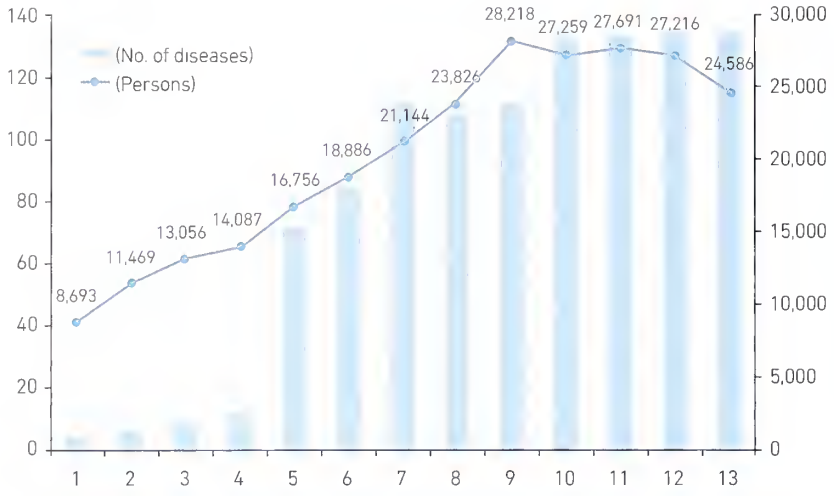


FIGURE 12. Increase in patients with a rare disease registered for the "National subsidy for Medical Expenses for Genetic and Rare Diseases" since 2001

expenses, and ventilatory support are provided for specific diseases (e.g., muscular diseases, multiple sclerosis, adrenoleukodystrophy, mucolipidosis, and hereditary ataxia).

FIGURE 13. Genetic and Rare Disease Center Website "Helpline" (http://helpline.nih.go.kr)

The "Genetic and Rare Disease Center" was established in 2006 to further assist patients and their families. The Center produces and distributes information about rare diseases via publications and a website (http://helpline.cdc.go.kr). The website is frequently visited by medical professionals, patients, and the public and provides general information (epidemiology, diagnosis, treatment, etc.) on 1,031 rare diseases as well as on regional rare disease hospitals, contact addresses of patient-support organizations, and information about specific diseases.

The Center funds a regional hospital network for genetic and rare diseases in four provincial areas (Chonnam, Chungnam, and Kyongnam/Pusan, Daegu/Kyonpook). This network is dedicated to organizing regional hospitals, improving genetic counseling, and educating the public.

A central control system for pulmonary rehabilitation of patients with a rare disease was started in 2013. A ventilator was used in the homes of 1,655 patients and home visits for 1,899 patients were carried out. From these results, high quality medical care services for patients who have been unable to receive proper management because of poor affiliation will be provided, and a local hospital professional cooperation system is planned.

Many rare diseases, particularly extremely rare diseases, are not properly diagnosed; in this context, many patients suffer from financial difficulties and do not understand their disease. The Genetic and Rare Disease Center has attempted to establish a diagnostics system in 2012 for patients with extremely rare diseases by providing a suitable disease cord and support in this program. The list of target diseases supported by the genetic diagnostic program for rare diseases is shown below.

Seoul National University and Younsei University Hospital corporately operate this genetic diagnostic program for rare diseases.

The Korean Mutation Database (KMD, http://kmd.cdc.go.kr) is a country-specific database of human gene mutations that was established in September, 2009. The KMD consolidates genetic disease information in Korea. The KMD contains 1,654 mutations and 4,143 individual patients with 245 genes. We collected mutation data from diagnostic laboratories and published studies over recent decades in Korea. The KMD has been open to the public without charge for searches, and it is open to individual researchers to

register mutation data. Thus, researchers have

Target Diseases	Genes	Remarks
ARC syndrome	VPS33B	Single
GM1 gangliosidosis	GLB1	Single
Leri-Weill Dyschondrosteosis	SHOX	Single
Short-chain acyl-CoA dehydrogenase deficiency	ACADS	Single
Acute intermittent porphyria	HMBS	Single
Rubinstein-Toybi syndrome	CREBBP	Single
Methylmalonic acidemia	MUT	Single
Ataxia-telangiectasia	ATM	Single
Miller-Dieker syndrome	LIS1	Single
Sandhoff disease	HEXB	Single
Sotos syndrome	NSD1	Single
Angelman syndrome	AS/PWS	Single
Permanent neonatal diabetes mellitus	KXNJ11, INS	Single
Systemicprimary carnitine deficiency	SLC22A5	Single
Zellweger syndrome	PEX1	Single
CHARG syndrome	CHD7	Single
Huntington diseasep-like 2	JPH3	Single
Holt-Oram syndrome	TBX5	Single
Familial amyotrophic lateral sclerosis	13 genes	Multi
Long QT syndrome	13 genes	Multi
Brugada syndrome	9 genes	Multi
Familial hypertrophic Cardiomyoparhy	31 genes	Multi
Familial dilated cardimopathy	47 genes	Multi
Hereditary PPGL syndrome	10 genes	Multi
Limb-girdle muscular dystrophy	20 genes	Multi
Fanconi's anemia	16 genes	Multi
Thoracic aortic aneurysms/Aortic dissection	12 genes	Multi
Ehlers-Danlos syndrome, types I and II	COL5A1/2	Multi

TABLE 4. The list of target diseases supported by the genetic diagnostic program for rare diseases

immediate access to mutation data, including gene and protein information. Our aim was to provide organized and curated information for clinicians and researchers who are interested in genetic diseases.

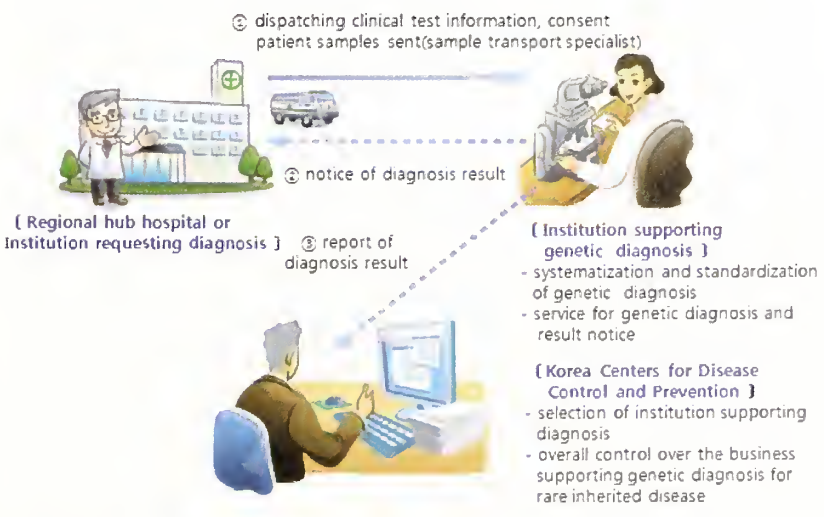


FIGURE 14. Flowchart of target diseases supported by the genetic diagnostic program for rare diseases

This database will be useful not only for researchers in Korea but also for those in countries with similar ethnic backgrounds.

Research into rare diseases is badly needed, as many patients lack a proper diagnosis and most are left without effective treatment. It is also an area in which experts are rare.

Increasing the number of diagnostic tests for patients suffering from rare diseases requires accelerating the identification of yet unknown critical genes and improving identification of gene defects. These tasks are critical to increase knowledge of the pathophysiology and natural history of rare diseases, to identify potential therapeutic targets, discover new biomarkers, and define appropriate surrogate end-points to adequately evaluate treatments and therapies. Therefore, the Genetic and Rare Disease Center is establishing clinical networks for rare diseases to collect clinical data on patients, increase knowledge of pathophysiology and the natural history of rare diseases, and to diagnose rare diseases. A clinical research network for histiocytosis, amyloidosis, and Crohn's disease was established in 2013. The Genetic and Rare Disease Center joined the International Rare Diseases Research Consortium to form a global network.

Future Plans

The support program for patients will continue to expand coverage. We will continue to recruit participants for the congenital malformation registry. To emphasize collaboration in rare disease research, we are establishing and managing clinical research networks on rare diseases, the rheumatic and musculoskeletal systems, genetic (inherited) arrhythmia disorders, neuromyelitis optica disorders, and inherited arrhythmia disorders.

Enforcement of the Bioethics Infrasturcture

Administration of the Bioethics and Safety Act

The Bioethics and Safety Act, which seeks to make progress in biomedical technology sustainable by prohibiting human cloning and providing guidelines for embryo research and genetic tests, has been in effect since 2005. The Act was revised in February 2012 to include all human research, and the revised Act has been enforced since February 2013.

The Division of Life Science Research Management enforces the Act by supervising embryo and somatic cell nuclear transfer (SCNT) research and by registering fertility clinics, embryo research institutes, corporations performing genetic tests, gene therapy clinics, and institutional review boards. We have sought to increase bioethics awareness to establish an infrastructure in which biomedical technology can progress, without harming human dignity, to improve national health and quality of life. To achieve this mission, we have continued to inspect fertility clinics, embryo research institutes, and genetic testing companies and have developed appropriate guidelines.

The Act defines six types of authorized research institutes or clinics, including fertility clinics providing *in vitro* fertilization services, embryo research institutes using surplus human embryos obtained from fertility clinics, SCNT (and parthenogenesis) embryo research institutes, corporations providing human genetic testing services, biobanks (previously gene banks) preserving and distributing human biospecimens, and gene therapy clinics. We authorized 40 clinics/institutes/corporations in 2013, after inspection or paper reviews of their facilities, equipment, employment practices, and institutional review boards. The numbers of registered clinics/institutes/corporations at the end of 2013 are shown below.

Oversight Activity

To improve the transparency of embryo production, research, and preservation, we inspected 32 authorized fertility clinics, 13 biobanks, and five corporations providing human genetic testing services in 2013. These inspections identified the need for the education of individuals in charge

TABLE 4. Numbers of authorized clinics and institutes at the end of 2013

Type	Number	Added in 2013	Closed in 2013
Fertility clinics		8	9
Embryo research institutes	52	-	12
Somatic cell nuclear transfer embryo research institutes	7	-	1
Corporations providing human genetic testing services	185	10	14
Biobanks	50	9	2
Gene therapy clinics	21	6	0
Total	465	33	38

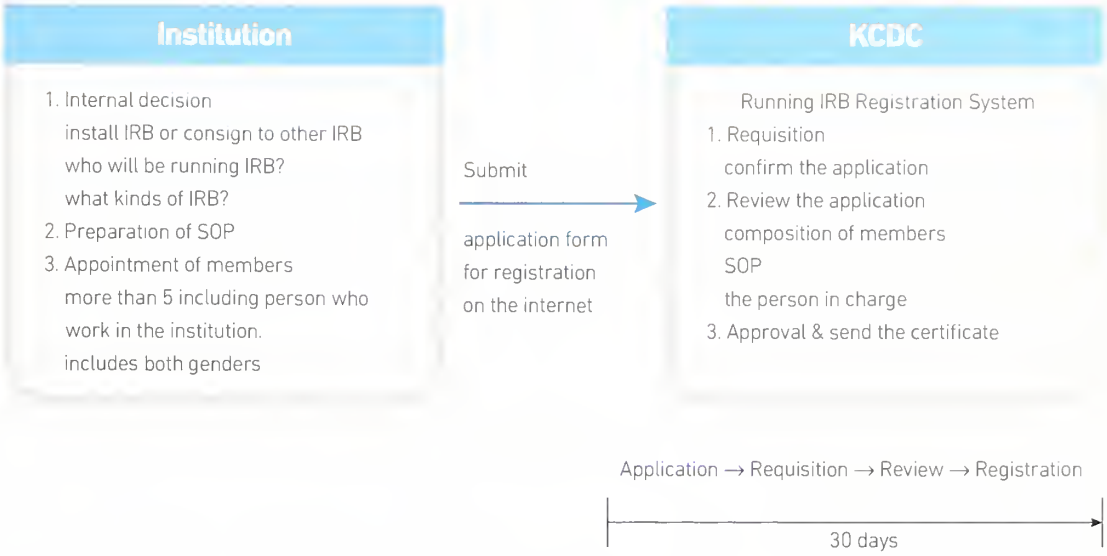


FIGURE 15. Procedure for institutional review board registration.

TABLE 5. Types of institutions that have institutional review boards in Korea in 2013.

Types of Institutions	Number of Institutions	%
Medical Institutions	280	53
Universities	155	29
Institutes	95	18
Total	530	100

TABLE 6. Types of institutional review boards registered in Korea in 2013.

Types of IRB (research fields)	Number of IRB*
Human subject research	373
Human biospecimen research	318
Fertility clinics	145
Embryo research	50
Somatic cell nuclear transfer embryo research	6
Biobanks	49
Human embryonic stem cell research	51
Total	992

* A total of 267 institutional review boards were registered to review more than two types of research fields.

of running institutional review boards in fertility clinics, as well as the education of people who explain informed consent for genetic testing. We also performed a 2013 survey of the current status of all corporations providing genetic testing and all biobanks to update the information.

Registration of Institutional Review Boards

Because the revised Act includes all human research, the institutional review board was introduced as a self-regulatory system. We established and managed an on-line system to apply for registration of an institutional review board. The types of institutions that have institutional review boards in 2013 are shown Table 1.

Information Service for Bioethics

We have set up two websites that have provided bioethics information since 2010. The Bioethics information website (bioethics.cdc.go.kr) provides authorized information, including news and guidelines. The genetic testing information website (genetest.nih.go.kr) provides scientific facts about genetic testing services. We developed on-line educational content in 2013 to provide information about the revised Act. The on-line content consists of four courses: human subject research and human biospecimen research, fertility clinics, biobanks, and genetic testing. The courses will be open at the KCDC education site (edu.cdc.go.kr) on May 1, 2014.

Institutional Review Board

We have been administering the KCDC institutional review board for intramural research on human subjects and biospecimens. We have convened institutional review board meetings bimonthly. Seventy-four research projects were reviewed during 12 meetings in 2013. According to the Act, we revised the SOP for the KCDC institutional review board. To improve awareness on the part of researchers and institutional review board committee members about bioethics, we held two education courses with the KOHI in 2013.

Clinical Research Support Program: National Clinical Research Coordination TF (NCRC)

Clinical research is research on patients, as well as various studies on prevention, early detection, and diagnosis of diseases and medical treatment. Clinical research advances health and medical care technology and provides clinical trial-based evidence to help physicians make decisions about healthcare services. As healthcare services should be offered based on evidence, standards for international clinical research have been instituted and have been developed on an ongoing basis. Thus, the need for efficient data management and conducting transparent clinical studies has increased. To respond to this need, the National Clinical Research Coordination TF (NCRC) was established in 2008 at the KNIH, and the NCRC has been operating the Clinical Research Support Program. This program is designed to share information about clinical research to be conducted in Korea and to support domestic research centers in conducting further clinical studies by providing management systems and training programs.

The objectives of the Clinical Research Support Program are to: (1) secure transparency and ethics of clinical research; (2) improve the quality of clinical research by providing a management system and training program for researchers; and (3) develop medical care services based on evidence by revitalizing clinical studies. Thus, the NCRC has three main objectives: (1) to operate the Clinical Research Information Service (CRIS), (2) provide the Clinical Research and Trial Management System (iCReaT), and (3) offer a Clinical Research Education Program.

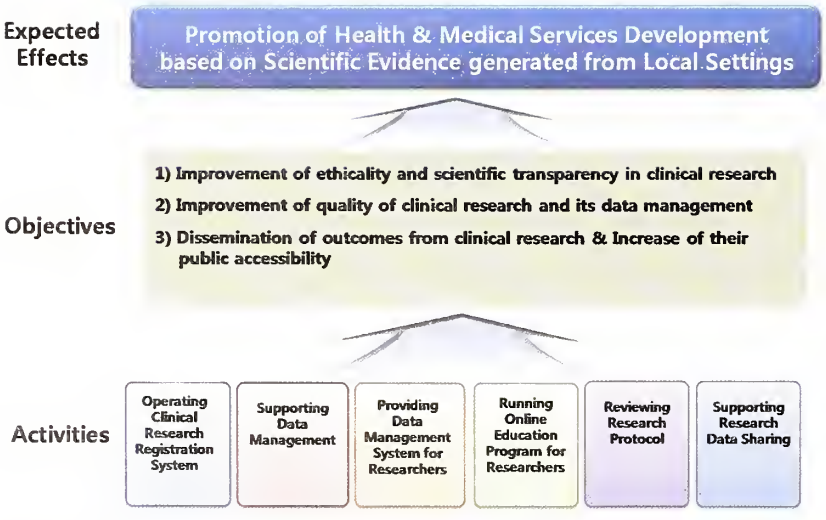


FIGURE 16. National Clinical Research Coordination TF (NCRC)>

CRIS

The CRIS (http://cris.nih.go.kr) is a non-profit online registration system for clinical studies, and it became the 11th Primary Registry of the WHO International Clinical Trials Registry Platform (ICTRP) in May, 2010. The main aims of the CRIS were: (1) to improve transparency, accountability, and ethics of clinical research conducted in Korea; and (2) to establish a medical research infrastructure based on scientific evidence. We have consistently registered clinical studies since May 2010 and have made researchers more aware of the importance of registering clinical research through various activities such as (1) amending "The Regulation for Management of Health and Medical Technology R&D Projects" (effective February 13, 2012), which specifies that it is mandatory to register clinical studies as designated by the Minister of Health and Welfare; (2) sending an information

letter to institutional review boards stating this amendment of the regulation; (3) introducing CRIS to researchers at relevant forums; and (4) publishing articles in newsletters and a magazine published by the KNIH. In addition, we started the CRIS mobile service in April 2013. Through these activities, the rate of registering clinical studies in the CRIS has increased continuously, and 962 studies have been registered in the CRIS as of December 31, 2013. The information entered into the CRIS is transferred to WHO ICTRP once per month and is made available to the worldwide public.

iCReaT

The iCReaT (http://icreat.nih.go.kr) is a web-based clinical research management system. It provides management programs relating to protocols, patients, data, and reporting. We also support iCReaT users to apply the system, and to improve clinical data quality, by giving them advice on eCRF design and protocol development. The iCReaT was developed from July, 2009, to September, 2010, and was opened to domestic researchers in October, 2010. We have actively publicized the iCReaT to researchers and have conducted both regular and study-based training for those who want to use the iCReaT. The training program consists of a 1-day basic course and 4-day advanced course. The iCReaT has been used for 42 studies since October 2010 and approximately 750 researchers have been trained to manage clinical data using the iCReaT (as of December 31, 2013). We have signed MOUs with four major hospitals in Korea (Ajou University Medical Center, Seoul Asan Medical Center, Seoul National University Bundang Hospital, and Yeungnam University Medical Center) to build a cooperative relationship for the use of iCReaT. Furthermore, the use of iCReaT became mandatory for certain types of clinical studies (i.e., stem cells) designated by the Minister of Health and Welfare based on the 2013 grant guidelines of the Korea Health Industry Development Institute.

Clinical Research Education Program

The NCRC has developed an online education program (http://edu.cdc.go.kr) intended to help researchers design and conduct clinical research more efficiently. The program consists of three courses: (1) clinical research and ethics, (2) clinical research design, and (3) sample size and power calculations. A total of 9,904 researchers were registered for these courses during 2013, and 7,097 of them completed the courses.

We will establish infrastructure for the virtuous circle of medical science knowledge and resources through a year-round supporting system including CRIS and iCReaT to manage government-funded clinical studies more efficiently. As grant guidelines have made it mandatory to register clinical studies with the CRIS and to use the iCReaT for some clinical studies, we expect that more clinical studies will be entered into our system. We will continuously update our system and keep it performing at its best. We will establish a more concrete legal basis for registering clinical studies, which includes more clinical studies and requires registration not only of information at the commencement of the research but also of the results upon completion.

National Center for Medical Information and Knowledge (NCMIK)

The plan to establish the NCMIK originated from the following background.

- As the output of publicly funded research is a public asset, and awareness of this has increased recently, it has been recommended that access to the output of government funded research be made readily available to the public and that infrastructure for this access be established.
- Because evidence-based medicine has been emphasized in providing health services and developing public health policy, the number of clinical studies has increased and a vast amount of medical information and research data have been produced.
- An institute or organization must be established that operates an integrated management system for medical academic information to improve access to medical science information

in practical settings because the demand for the latest research information has increased, given the provision of evidence-based health services.
- Strategic investment by establishing mid-term and long-term transgovernmental medical health R&D plans has increased and efficient management of the research output is required to foster future growth at the national level.
- A responsible facility is needed to collect, integrate, and analyze biomedical information as a national knowledge resource to facilitate personalized medicine.

The mission of the NCMIK is to reproduce new knowledge from medical science information by providing support services to produce, process, and disseminate the information. Thus, the NCMIK has the following objectives: improve publicity of the output and resources produced from medical science research; manage and disclose data generated by the domestic medical science field; manage the medical science research resources; and facilitate the use of these resources.

The detailed objectives are described as follows:
- The **National Medical Library** manages and provides reports and articles in the medical science field at the national level
  - Collects, integrates, informalizes and provides medical science literature at the national level
  - Applies a MeSH classification system to conduct integrated management and provide core information
  - Establishes Korea PubMed Central (PMC) as an open repository to share the national R&D output
- **Management of medical science knowledge**: systematically collecting and providing medical science knowledge and resources
  - Provides a platform and information to share a large amount of genomic data
  - Establishes and shares an information system to disseminate research output such as information about genomic polymorphisms or mutations, tentatively named the Clinical & Omics Data Archive (CODA)
  - Standardizes terms and adopts the Metadata system to share research resources
    ※ Metadata are the data characteristics. Metadata must be processed after being defined at the data collection stage to share all of the data,
  - Operate and support a research data management system to systematically produce research resources

The major activities of the NCMIK in 2013 were as follows:
- CODA assigned to NCMIK

The Ministry of Health and Welfare instructed the NCMIK to operate CODA in January, 2013
- Setting up the NCMIK information system

The Information system was set up on June 10, 2013. The system includes the NCMIK portal website, Korea PMC, MeSH indexing, and management of literature, reports and articles in the medical science field.
- Conducted a MeSH indexing pilot project

The purpose of this project was to improve use of domestic academic data in the medical science field by standardizing the classification system for these data. The project included a training program for MeSH indexers conducted May 5– July 18, 2013.
- Completed construction of the NCMIK building

Construction was completed in November, 2013, and NCMIK will officially open in March, 2014, after a trial run of the information system.
- Collected domestic healthcare journals

We collected approximately 4,157 volumes from 322 journals among 600 medical science journals currently being published in Korea

Our plans for 2014 are described as follows:
- Opening ceremony for the NCMIK building

The ceremony will be held at the National Biobank auditorium on March 27, 2014. A symposium will be conducted on strategies for information sharing, and on vitalization of public access to the medical science information and research data.
- Management of research information and data in the medical science field

We will establish an infrastructure to legislate research information sharing. We will develop a system and guidelines for managing and providing medical information and research data
- Cooperate with relevant organizations

FIGURE 17. Tape cutting ceremony at the National Center for Medical Information and Knowledge opening

We will conclude an agreement with relevant organizations for collecting domestic journals in the medical science field.

- Collection of domestic journals in the medical science field

We will continuously collect volumes issued by 600 journals and expect approximately 460 journals to be collected by 2014

Accomplishments

Publications

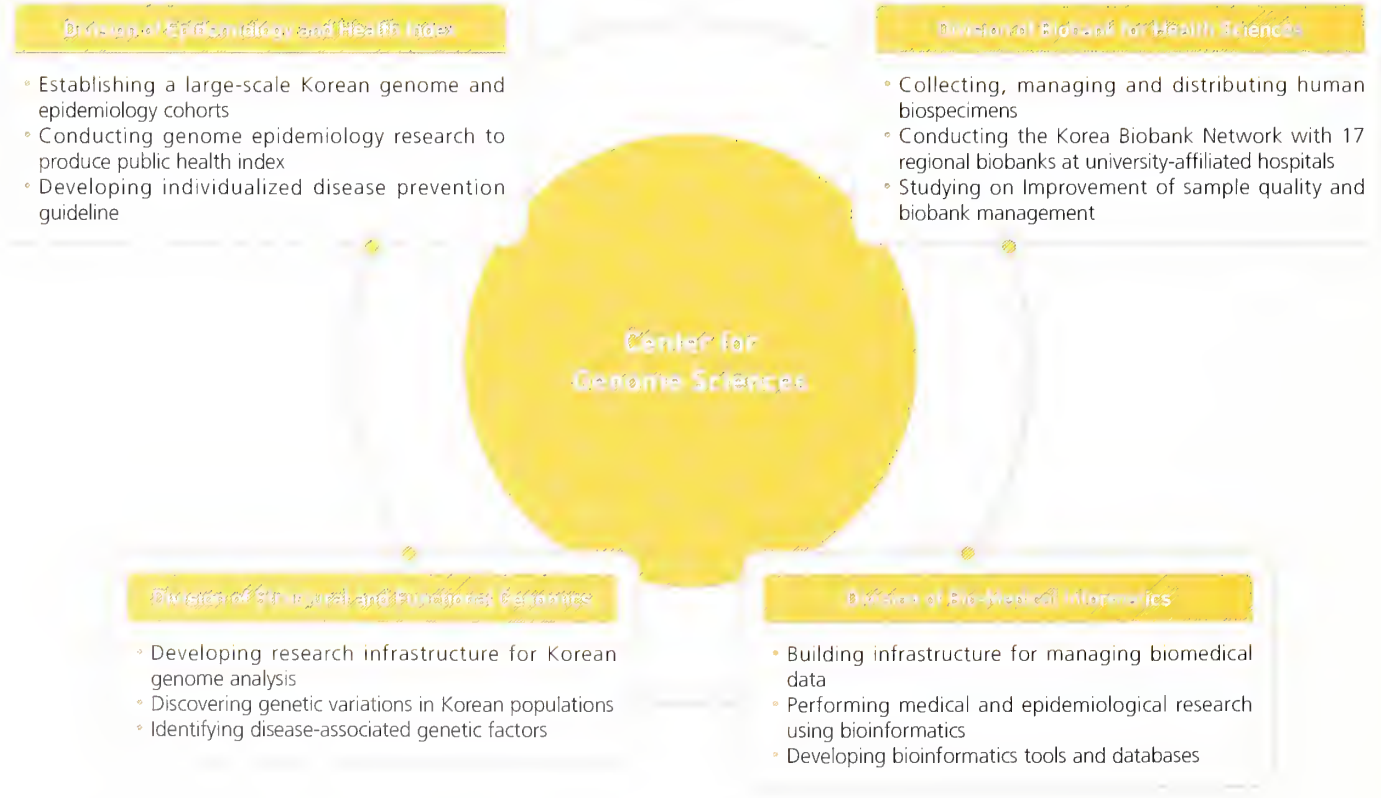
1. BO Choi et al. Clinical and histopathological study of Charcot-Marie-Tooth neuropathy with a novel S90W mutation in BSCL2. Nuerogenetics. 14:35-42 (2013).
2. SJ Park et al. TRAIL regulates collagen production through HSF1-dependent Hsp47 expression in activated hepatic stellate cells. Cellular Signalling. 24(7):1635-43 (2013).
3. HM Woo et al. Whole-exome sequencing identifies MYO15A mutations as a cause of autosomal recessive nonsyndromic hearing loss in Korean families. BMC Medical Genetics. 14:72 (2013).
4. Yu, Kyung-Rok et al. HMGA2 regulates the in vitro aging and proliferation of human umbilical cord blood-derived stromal cells through the mTOR/p70S6K signaling pathway. Stem Cell Research 10(2):156-165 (2013).
5. Kim S *et al.* Cadmium induces neuronal cell death through reactive oxygen species activated by GADD153. BMC Cell Biology. 2013;14:4-13.
6. Jo C and Koh YH. Cadmium induces N-cadherin cleavage via ERK-mediated γ-secretase activation in C6 astroglia cells. Toxicology Letters. 2013; 222:117– 121.
7. Lee SK *et al.* S1 pocket of glutamate carboxypeptidase II: A new binding site for amyloid-β degradation. Biochemical and Biophysical Research Communications. 2013; 438: 765–771
8. Yoon SM *et al.* SUMO1 modulates Aβ generation via BACE1 accumulation. Neurobiology of Aging. 2013;34: 650-662.
9. Nam-Kyoo Lim et al., Predicting the risk of incident hypertension in a Korean middle-aged population: Korean Genome and Epidemiology Study. The Journal of Clinical Hypertension. 2013. 5(15)344-349.
10. Hyung jin kim et al., Conditional deletion of Pten leads to defects in nerve innervation and neuronal survival in inner ear develpoment. Plos One. 2013. 8;2:e55609:1-11
11. Min-ju Kim et al., Association between shift work and obesity among female nurses: Korean Nurses's Survey. BMC public health. 2013. 13: 1204
12. Kuk Hui Son et al., Association between fasting blood glucose and carotid intima-media thickness of polycystic ovary syndrome patients with normal glucose tolerance. Diabetes Care. 2013. 36:e66-e67.
13. JS Choi *et al.* Effects of excess dietary iron and fat on glucose and lipid metabolism. J NUTR BIOCHEM. 24(9):1634-44(2013).
14. Ji Yeon Kim *et al.* In vivo activating transcription factor 3 silencing ameliorates the AMPK compensatory effects for ER stress-mediated β-cell dysfunction during the progression of type-2 diabetes. CELL SIGNAL. 4(4):179-186(2013).
15. Han Byul Jang *et al.* Association Between Serum Vitamin D and Metabolic Risk Factors in Korean Schoolgirls. Public Health Res Perspect, 4(4):179-186(2013).

Center for Genome Sciences

The Center for Genome Sciences (CGS) strives for predictive, preventive, and personalized medicine through Korean genomic research on major chronic disorders, and the establishment of a research infrastructure. The CGS is composed of four divisions including the Division of Epidemiology and Health Index, the Division of Bio-Medical Informatics, the Division of Structural and Functional Genomics, and the Division of Biobank for Health Sciences. These divisions are conducting four research projects: the Korean Genome and Epidemiology Study (KoGES), the Korean Genome Analysis Project (KoGAP), the Korean Epigenome Project (KEP), and the Korea Biobank Project (KBP).

The KoGES is collecting human biospecimens and epidemiological and bio-medical data from voluntary nationwide cohorts. These bio-resources are used for the KoGAP and are distributed to Korean bio-medical researchers. The purpose of the KoGAP is to identify genetic risk factors for lifestyle-related diseases (diabetes, hypertension, osteoporosis, obesity, and metabolic syndrome). The KEP aims to provide a comprehensive epigenomic map of Korean chronic disease-related target tissues. The KBP is a project that collects, manages, and distributes human biospecimens and related information. These bioresources are collected through various cohorts and the Korea National Health and Nutrition Examination Survey.

These projects ultimately promote public health by enhancing bio-medical research.



KoGES (Korean Genome and Epidemiology Study)

Chronic diseases, such as cancer, heart disease, and diabetes, are the leading causes of all death in Korea and are influenced by complex genetic and environmental factors. To improve evidence-based prevention strategies for chronic diseases and provide infrastructure for biomedical research, the division of Epidemiology and Health Index of the KCDC has been conducting a large-scale cohort study since 2001 called the Korean KoGES.

The KoGES is a prospective cohort study of the Korean population. The objective was to collect various individual health and lifestyle-related data

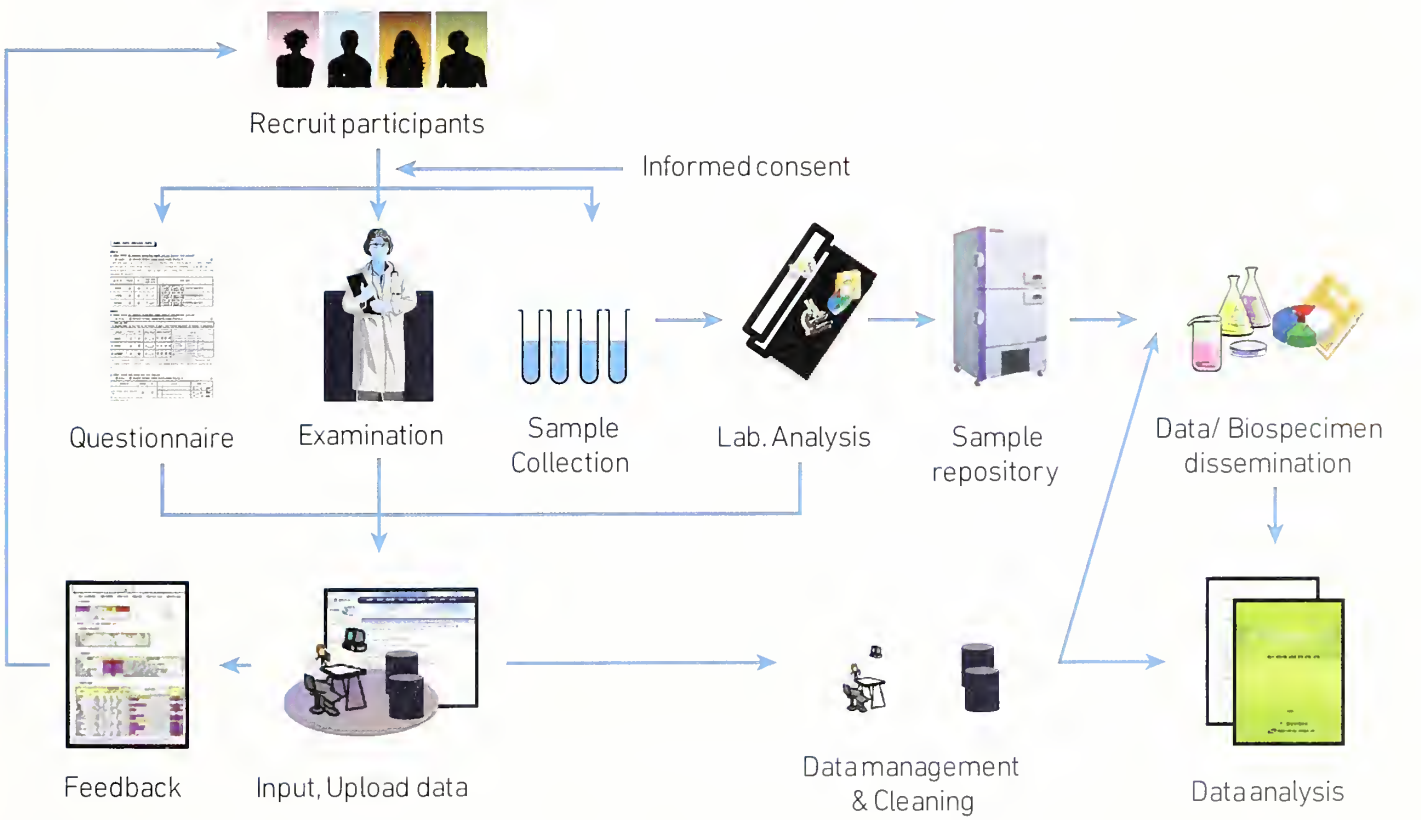


FIGURE 1. Korean Genome and Epidemiology Study procedure

as well as biological samples (blood and urine) by conducting interviews and physical examinations of study subjects (Figure 1). Since 2001, KoGES has been expanding and diversifying the study hypothesis and is currently composed of seven different projects, which include general population-based cohorts and gene-environmental model cohorts. The cohort studies provide a detailed assessment of exposure and outcomes, offering the most comprehensive approach to delineating genetic and environmental predisposition and their interactions (Tables 1 and 2).

COHORT STUDIES IN THE KOGES

Community-based cohorts

A general population cohort was established in Ansan, a small urban city, and Ansung, a rural city. Enrollment for the study was based on community characteristics and on the efficiency of recruiting representative samples of the target population.

A total of 10,038 subjects (age, 40–69 years), who submitted informed consent, were examined in the baseline survey between June 2001 and January 2003. Follow-up examinations have been conducted at 2-year intervals since 2003. The overall follow-up rates at the first 2-year follow-up examination in 2003–2004 were 80.5% in Ansan and 92.6% in Ansung, excluding those who died during the follow-up period and those who refused to participate. The subsequent follow-up rates at the third, fourth, and fifth repeated surveys were 80.1%, 70.5%, 64.2%, 65%, and 60.8% in Ansan and 91.3%, 79.2%, 68.4%, 67.8%, and 63.5% in Ansung, respectively (Figure 2). We expect that repeated follow-up survey data on this 10-year-old large population-based cohort will provide extremely valuable information for investigators in the epidemiology and genomic communities. As the number elderly participants in the baseline population increases, we will include some in-depth examinations related to aging, such as brain MRI, and a cognitive function questionnaire. These data will enable us to study age-related diseases and health issues.

About 28,500 participants in six different areas have been surveyed to recruit subjects living in rural areas, and 14,500 have been recalled for the first round and 6,200 for the second round of the ~3 year repeated follow-up.

Large-scale health examinee cohort

We have been also managing multicenter cohorts to expand the base size of the KoGES cohort. Participants were recruited from healthy patients who visited the hospital for regular health check-ups, to collect representative samples of the general population from urban areas. The centers that participate in the KoGES large-scale cohort project are based in hospitals located across the country, including urban and rural areas. Members of the general population aged 40–69 years are the major participants. At the end of 2013, about 180,000 subjects residing in urban areas had been enrolled in the base survey.

Gene-environment model cohort

Cohorts for several models have been established to elaborate the chronic disease risk factors between the environment and the genome and to assess genetic susceptibility based on familial studies.

COHORT STUDY OF MARRIAGE-BASED IMMIGRANTS

This cohort is comprised of immigrant women from Southeast Asian countries such as Vietnam and Cambodia and their families. Recruitment started in 2006, and 7,500 subjects have been enrolled as of 2013. The first round of active repeated follow-up surveys was initiated in 2012.

KOREAN EMIGRANT COHORT STUDY

This cohort consists of emigrant Koreans who have been residing in foreign countries including Japan and China for at least 15 years and includes natives of those countries as controls. Recruitment commenced in 2005. In total, 1,063 subjects were enrolled in Japan and 2,485 in China for the baseline survey. Among these, 773 (72.7%) subjects in Japan and 1,094 (45%) in China have been re-examined at the first 3-year follow-up. A follow-up survey has been continued only for the participants in Japan. The secondary follow-up rate was 52% and the tertiary follow-up was completed in 2013 at 49%.

INTERNATIONAL COLLABORATION STUDY II

This cohort was constructed as a country-of-origin control group for marriage-based immigrants and to identify chronic disease risk factors for metabolic disorders, diabetes, and hypertension, which are attributable to genetic and environmental differences between Korea and Southeast Asian

countries. Participants included family members of immigrant women who remain in their native countries and unrelated women in those countries whose age range was similar to that of the immigrants. As of 2012, 3,537 participants have been recruited from Vietnam.

TWIN AND FAMILY COHORT STUDY

Studies involving twins are a powerful research design to identify interactions between genetic and environmental factors for various diseases. A total of 3,500 monozygotic twins and their families have been recruited for the baseline survey as of 2012, and 2,076 have been reexamined at the first follow-up. The second round of repeated follow-ups is currently under way.

Quality assurance and control strategies for the KoGES

Our survey procedure is described in Fig. 1, and more detailed information about the cohorts and the survey items are available at the KoGES of KNIH Homepage (**http://www.nih.go.kr/NIH/eng/main.jsp**). We have been developing and implementing strategies to manage data quality control and standardization for issues pertaining to questionnaire surveys, physical examinations, and bio-specimen collections. The computer-assisted interviewing system (CAPI) has been operating since 2010 for the urban multicenter health examinee cohort and is used to conduct surveys and standardize survey data. The interviewers and support staff receive training two or three times

TABLE 1. Korean Genome and Epidemiology Study questionnaire items

Classification	Items
General characteristics	Sex, age, marital status, educational level, job, social and economic level, family status, place of residence, etc.
Past medical history	Past disease history, family history of diseases, surgical record, intake of nutrient supplements
Smoking & drinking habits	Smoking status, starting age of smoking, duration and amount of smoking, Drinking status, duration and amount of drinking, etc.
Sleeping, physical activities	Sleeping hours, physical activity level(during working and leisure time)
Dietary questionnaire	Food frequency questionnaire, dietary habits, 24hr recall
Reproductive factors	Menstruation, pregnancy, childbirth, breast-feeding history, intake of birth control pills
Social & psychological factors	Stress, social network, social support

TABLE 2. Clinical measurement

Classification	Items
Anthropometry	Height, weight, waist circumference, hip circumference
DM screening	FBS, OGTT, blood insulin level
Vital signs	Blood pressure, pulse, EKG
Body composition	Body fat assessment
Blood and urine test	CBC, Protein, Albumin, BUN, Creatinine, Calcium, Bilirubin, AST, ALT, r-GTP, Total cholesterol, HDL-cholesterol, Triglyceride, Sodium, Potassium, Chloride, CRP, Routine Urine test
Special examination	Bone density, X-ray, PFT etc.

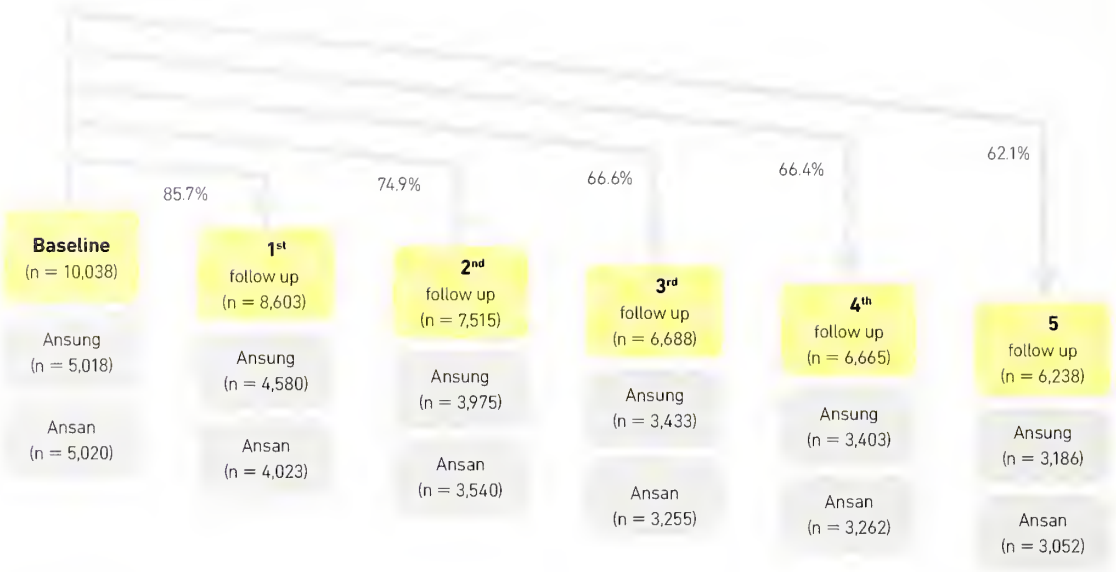


FIGURE 2. Follow up rate of the repeated survey in the community-based Ansan, Ansung cohort

per year about interview techniques, data entry methods, bio-specimen handling, measurement of anthropometric characteristics, and the informed consent process to ensure standardization of the collected data. Additionally, KoGES protocols, such as questionnaires, physician and nutrition examinations, and cohort epidemiology information systems were published (Fig. 2). Finally, KoGES projects have been implementing data release criteria and processing requests for data sharing for the Korean representative cohort infrastructure. Researchers are expected to submit a study plan with institutional review board approval to use the KoGES data.

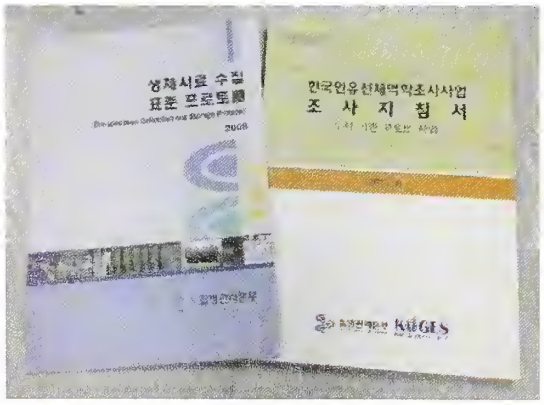


FIGURE 3. KoGES protocols and guidelines to standardize survey and examination method and procedures.

Major research theme of the KoGES

Metabolic diseases such as type 2 diabetes, hyperlipidemia, hypertension, and obesity are public healthcare issues. We are collecting data to understand the epidemiological features of Korean metabolic diseases: anthropometric traits (blood pressure, pulse rate, BMI, and waist circumference), clinical laboratory data (fasting glucose level, 1 and 2 h glucose levels after oral glucose tolerance testing, hemoglobin A1c, cholesterol levels, and triglycerides), questionnaire-based confounding factors (disease history, treatment status, and family history) and other environmental factors (smoking status, alcohol drinking habits, food preferences, physical activity, income, and education). As all KoGES cohorts have reached the follow-up re-examination stage, KoGES resources have several advantages, such as detailed exposure assessments, cause-specific morbidity and mortality estimations, minimization of bias to verify cause-effect relationships, and multiple-endpoint analysis. Additionally, the KoGES epidemiology resources can be linked to high-throughput genome variation

data, such as single nucleotide polymorphisms, copy number variations, and bio-specimens, such as serum, DNA, and immortalized leukocyte cell lines. Researchers can utilize multi-dimensional and comprehensive approaches to identify the determinants and underlying mechanism of metabolic diseases using the KoGES cohort data.

Data sharing and collaborative research

The baseline and primary follow-up data from the community-based cohort studies were made available to research in 2005 and 2007, respectively. The secondary follow-up data on 2,135 parameters of 7,515 participants were made available in 2008. The process data for the nutritional survey were added to the shared data list in 2010. As of 2013, researchers benefited from data sharing from more than 100 research projects. In addition to these data, the center has been sharing certain resources including the food frequency questionnaire and its database with researchers who want to conduct their own nutrition surveys.

KoGAP (Korean Genome Analysis Project)

Recent advances in genotyping technologies and analytical methods have allowed the use of genome-wide association (GWA) studies to investigate genetic factors for controlling traits in the genome of a large population. The successes of GWAS in identifying specific genes that affect risk for common diseases are dramatic illustrations of how improved technology can lead to scientific breakthroughs.

The goals of the KoGAP are to identify genetic risk factors and their relationships with environmental factors that influence lifestyle-related complex diseases such as diabetes, hypertension, obesity, and metabolic syndrome.

We published several articles in last year. Highlights of the researchers' accomplishments for the last year include:

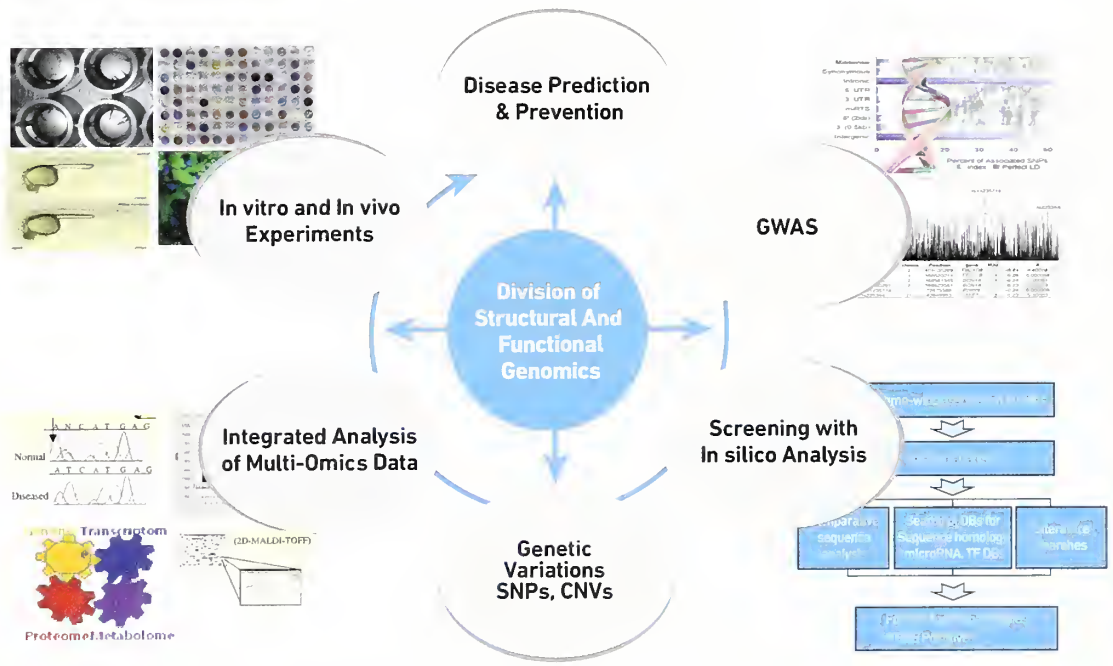


FIGURE 4. Division of Structural and Functional Genomics

- Identification of heart rate-associated loci and their effects on cardiac conduction and rhythm disorders. *Nat Genet*
- A large-scale association analysis that identified new risk loci for coronary artery disease. *Nat Genet*
- A genome-wide association study meta-analysis that revealed transethnic replication of mean arterial and pulse pressure Loci. *Hypertension*
- A meta-analysis that identified a MECOM gene as a novel predisposing factor for osteoporotic fracture. *Journal of Medical Genetics*
- KGVDB: a population-based genomic map of CNVs tagged by SNPs in Koreans. *Bioinformatics*

The project consists of seven intramural studies:

- A study to analyze the Korean Reference Genome
- Genomic studies to identify genetic factors of metabolic diseases in the population-based cohorts
- Functional validation of disease-associated genomic markers using model organisms and omics data
- Construction of a prediction model based on missing heritability for complex diseases
- Genome-wide rare copy number variations (CNVs) association study with complex diseases
- Genomic association and functional identification studies for genetic factors of osteoporosis-related traits
- Establishment and confirmation of an epigenomic analysis platform using the disease discordant twin cohort

This project will determine the genetic bases for medically relevant traits and lifestyle-related complex diseases, establish databases for Korean genetic variations such as SNPs and CNVs, structural variation (SV), and insertions/deletions (indels), and facilitate research in the area of genomic science in Korea.

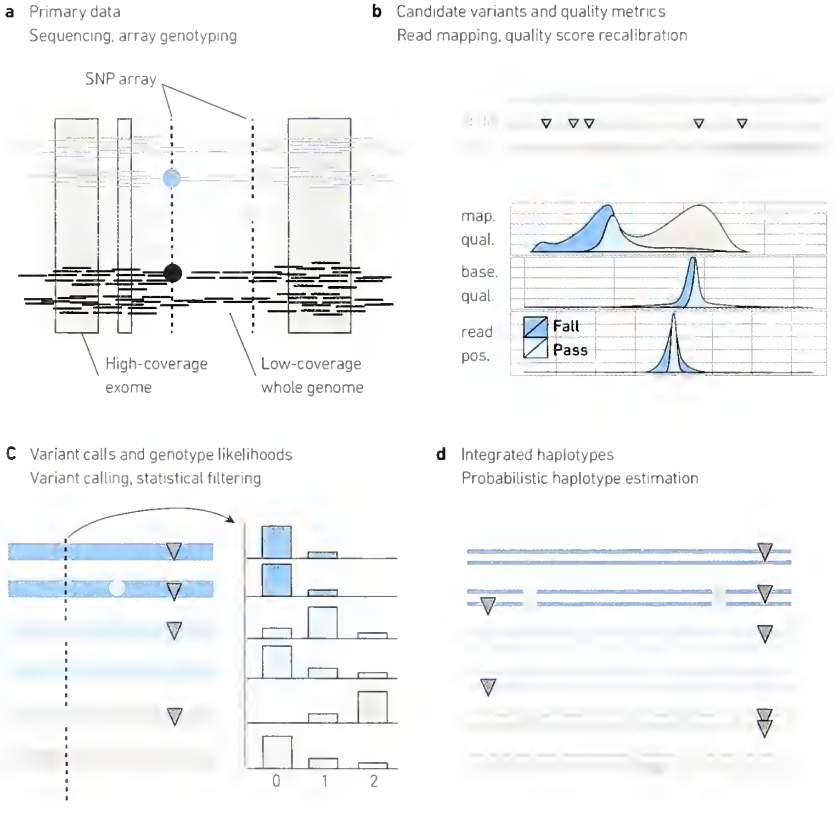


FIGURE 5. Constructing an integrated map of variation <1,000 genomes project, Nature Genetics 2012>

KRG(KOREAN REFERENCE GENOME)

Reference haplotypes of 400 Korean samples

Next generation sequencing (NGS) technology is becoming a powerful tool for discovering genetic variants across entire chromosomes in genomic studies. However, NGS is not feasible to apply in a large-scale population based genomic study due to its relatively high cost and high computing power. Imputation analysis has attracted attention as an alternative tool to comprehensively analyze all genetic variants in a large-scale population genomic study. In brief, imputation predicts untyped markers that exist in a reference panel not yet genotyped in previously genotyped microarray data. Untyped markers are predicted based on matched haplotype patterns between reference and genotype panels. Imputation analysis typically requires a reference panel with a large set of markers. Thousands of sequenced samples from the 1,000 genomes project are commonly used as the reference panel. The major advantage of imputation analysis is that it expands the set of markers for association mapping. Imputation analysis enables us to perform *in silico* fine mapping. Currently, 38 million SNP markers are available in the reference panel of the 1,000 genomes project.

The construction of a population-specific reference panel and an imputation-based

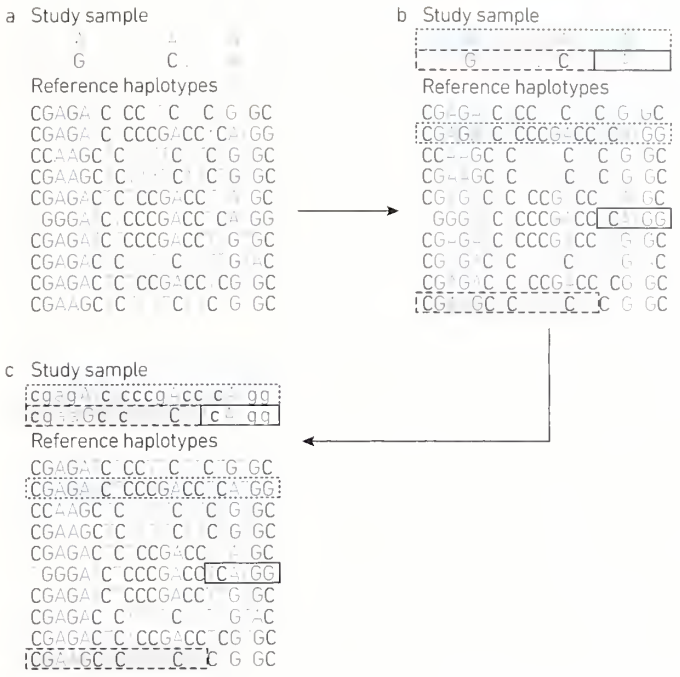


FIGURE 6. Imputation procedures

association study successfully identified previously hidden markers associated with various phenotypes. For example, Auer et al. (AJHG 2012) reported previously undiscovered variants associated with blood cell traits. However, the public resource of sequencing data did not include Korean samples. About 500 Japanese and Chinese samples are currently available as the East Asian reference panel in the 1,000 genomes project data (Table 1). Therefore, the Korean Reference Genome (KRG), in terms of the Korean Reference Haplotype (KRH), will be a powerful resource for genomic disease studies in the Korean population. The KRG project was initiated in 2012. The main goal of the KRH, as part of the KRG, was to develop a population-specific reference panel of Korean samples for imputation analysis. At the end of 2013, 400 samples (~20x depth) were sequenced and are currently under intensive analysis including quality control, variant calling, and haplotype phasing. Completion of the KRH and the opening to the public will occur at the end of 2014. The KRH of KRG will be a valuable resource and will be a great aid for imputation-based association analysis of all genetic variants across the whole genome of the Korean population.

GWAS

Recent advances in genotyping technologies and analytical methods have allowed the use of GWAS to investigate entire genomes in large populations for genetic factors controlling traits with significant

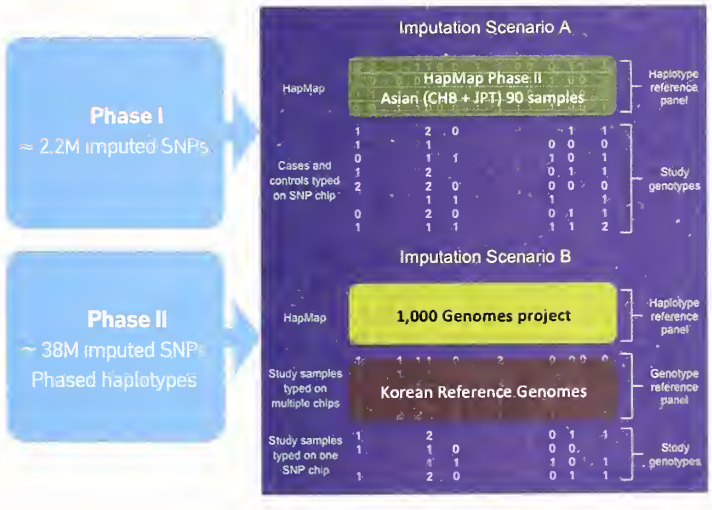


FIGURE 7. Imputation Scenario

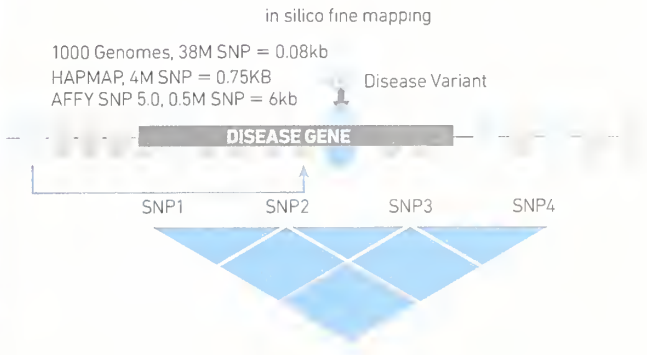


FIGURE 8. In silico fine mapping

TABLE 3. Sample information of 1,000 Genomes Project

Group	Population	Acronym	Number
European ancestry	Utah residents (CEPH) with Northern and Western European ancestry	CEU	100
	Toscani in Italia	TSI	100
	British from England and Scotland	GBR	100
	Finnish from Finland	FIN	100
	Iberian populations in Spain	IBS	100
West African ancestry	Yoruba in Ibadan, Nigeria	YRI	100
	Luhya in Webuye, Kenya	LWK	100
	Gambian in Western Division, The Gambia	GWD	100
	Mende in Sierra Leono	MSL	100
	Esan in Nigeria	ESN	100
Americas	African Ancestry in Southwest US	ASW	62
	African Caribbean in Barbados	ACB	100
	Mexican Ancestry in Los Angeles, CA	MXL	70
	Puerto Rican in Puerto Rico	PUR	90
	Colombian in Medellin, Colombia	CLM	89
	Peruvian in Lima, Peru	PEL	89
South Asian ancestry	Gujarati Indian in Houston, TX	GIH	100
	Punjabi in Lahore, Pakistan	PJL	100
	Bengali in Bangladesh	BEB	100
	Sri Lankan Tamil in the UK	STU	100
	Indian Telegu in the UK	ITU	100
East Asian ancestry	Han Chinese in Beijing, China	CHB	100
	Japanese in Toyko, Japan	JPT	100
	Han Chinese South	CHS	100
	Chinese Dai in Xishuangbanna	CDX	100
	Kinh in Ho Chi Minh City, Vietnam	KHV	100
Total			2,500

impacts on public health. Most GWAS have been conducted on subjects with European ancestry. Therefore, the discovery of new associations and validation of the genetic effects of loci previously reported in individuals of East Asian ancestry are warranted.

In this context, we conducted GWAS to explore the genetic basis of lifestyle-related complex diseases (such as type 2 diabetes, hypertension, obesity, dyslipidemia, and osteoporosis) and medically relevant traits (such as BMI, waist-to-hip ratio, height, systolic and diastolic blood pressure, pulse rate, measures of bone density, and circulating lipids, glucose, insulin, and liver enzyme levels) in the Korean population. The discovery and a subsequent replication study on genetic effects of variants were performed in tens of thousands of individuals taken from the Korea population and populations with Asian ancestry. We initially genotyped individuals with commercial genotyping arrays containing 0.5–1 million (M) SNPs. We performed imputation analysis on the genotyped information to expand the number of association mapping markers. Imputation constitutes analysis for estimating untyped markers that do not exist in genotyping arrays. For example, 0.5 M SNPs can be expanded to 38 M SNPs and the gain in information by imputation analysis is about 76 times, compared with the original information. We identified multiple genetic loci showing strong evidence of associations responsible for the variation in phenotypes in a series of association studies.

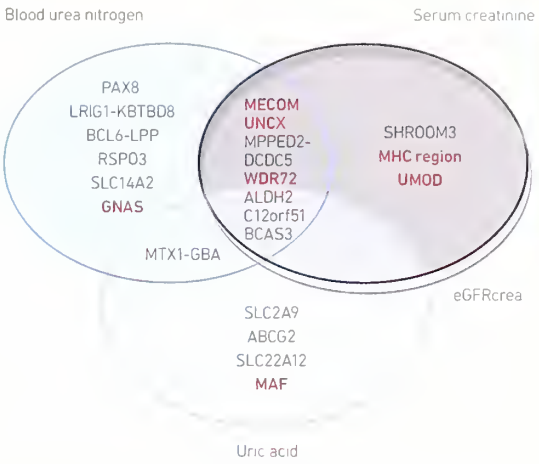


FIGURE 10. Pleiotropic associations of kidney function related traits

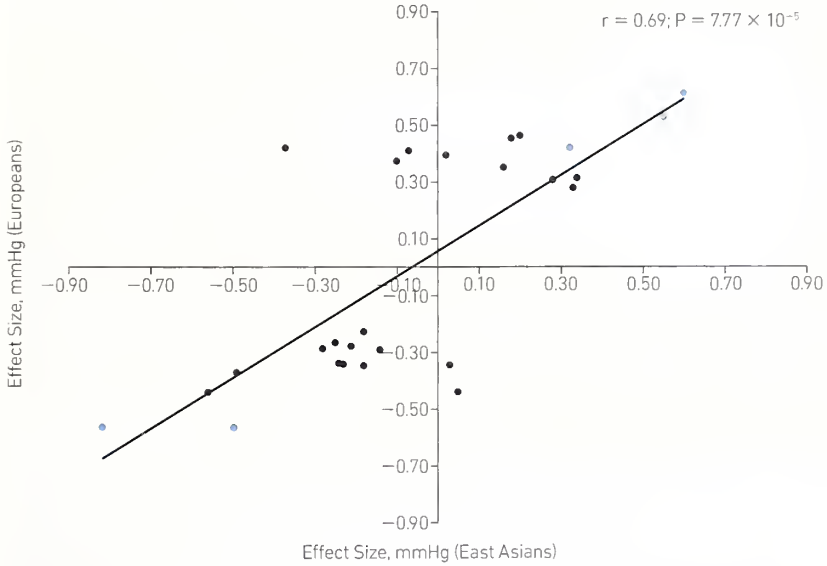


FIGURE 9. Trans-ethnic replicability of MAP-PP associated loci (East Asians vs Europeans)

We observed trans-ethnic replicability and pleiotropy in associated loci based on the GWAS results. If a locus presents consistent genetic effects across populations with different ancestry, this property of genetic effects can be called "trans-ethnic replicability". Trans-ethnic replicability is particularly important in human disease genetics. As a description of the genetic architecture of human diseases is the basis of the personal genome, personalized medicine, and public health genomics, the portability of findings across populations is an important issue for building a prediction model and prioritizing strategies to identify disease-associated loci in a specific population. Moreover, if the shared genetic component is common across populations, it suggests that trans-ethnic GWAS and fine mapping is a powerful tool to identify new associations. Some regions showed associations in a population-specific manner due to differences in genetic architecture among populations. We observed pleiotropic regions in which the locus was associated with multiple phenotypes. The pleiotropy of multiple phenotypes reflects a shared genetic pathway among phenotypes. Identifying pleiotropic

regions is valuable for understanding the underlying mechanism of diseases.

All summarized information from genomic variants will be accessible via a dedicated Korean genomic variants website.

STUDY OF STRUCTURAL VARIATION

Genomic structural variation (SV) is the structural variation in a DNA segment. It includes deletions, duplications, insertions/deletions (indels), inversions, translocations and CNVs. Some SVs are associated with human genetic diseases and disease susceptibility. Detecting SVs is more difficult than SNPs but recent advances such as NGS and comparative genomic hybridization array (aCGH) techniques have enabled the accurate identification of SVs. The purpose of our study was to elucidate SVs affecting quantitative traits (QTs) and complex diseases in the Korean population and to implement databases and SV-related applications. We have constructed a CNV database called KGVDB by collaborating with the Division of Biomedical Informatics at KNIH. In addition, we have implemented a family-based CNV association algorithm called PedCNV by collaborating with Chung-Ang University. The computational efficiency of PedCNV enables genome-wide association analysis, and we have shown that the proposed method outperformed existing approaches. The proposed method was applied to the CNV analysis of red-blood related phenotypes in Korean family-based samples and identified copy number deletions significantly associated with hematological traits. We set up a CNV analysis pipeline using whole exome sequencing data to detect the SVs affecting QTs. With this pipeline, we undertook the estimation of the CNV genotype, and 50% of the estimated CNVs were evaluated by customized arrays. Moreover, we suggested a combined workflow to estimate CNV genotypes using an existing CNV detection and association tool. We demonstrated that genotyping accuracy is highly reliable when the number of CNV calls is more than five. Disease association studies with large-size and small-size SVs such as indels and CNVs < 50 bp length will be conducted in 2014.

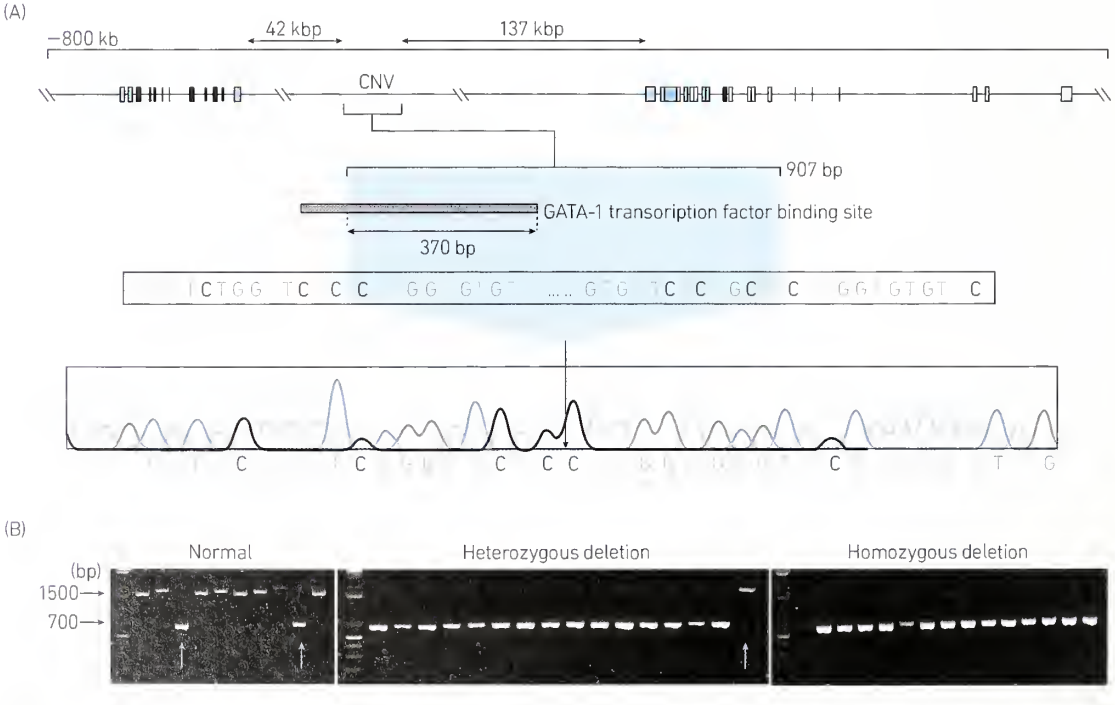


FIGURE 11. The significant copy number variation (CNV)region associated with hematological trait

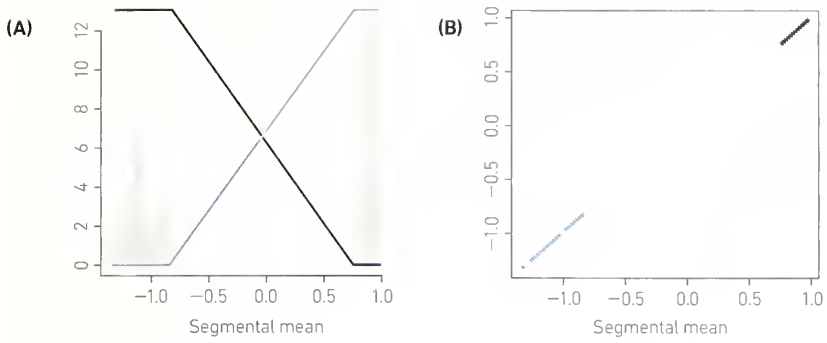


FIGURE 12. Clustering result from combined workflow for copy number variation (CNV) genotyping

FUNCTIONAL STUDIES OF GENETIC VARIANTS ASSOCIATED WITH HUMAN DISEASES AND TRAITS

Experimental validation

Since completion of the Human Genome Project, gene alterations such as SNPs and CNVs have received particular attention in the field of disease etiology. However, gene alterations associated with human diseases and traits based on statistical analysis should be validated and confirmed to determine biological relevance using the appropriate functional analyses.

First, candidate genetic variants related to diabetes, hypertension, obesity, and bone-related diseases were identified using epidemiological, clinical, and genotype data. Second, genetic variants using bioinformatics analyses such as comparative genomics, sequence homology, regulatory elements and literature studies were screened using various databases. Third, metabolomic data were produced using human serum and will be used for integrated analyses with other omics data including genomics, transcriptomics and epigenomics to determine the associations of genetic variants with the mechanisms of disease initiation and progression. Finally, functional validation of cell-based and model organism-based analyses including zebrafish using targeted genome editing and/or siRNA knockdown experiments will be helpful to elucidate the mechanisms of disease progression and disease diagnosis and treatment.

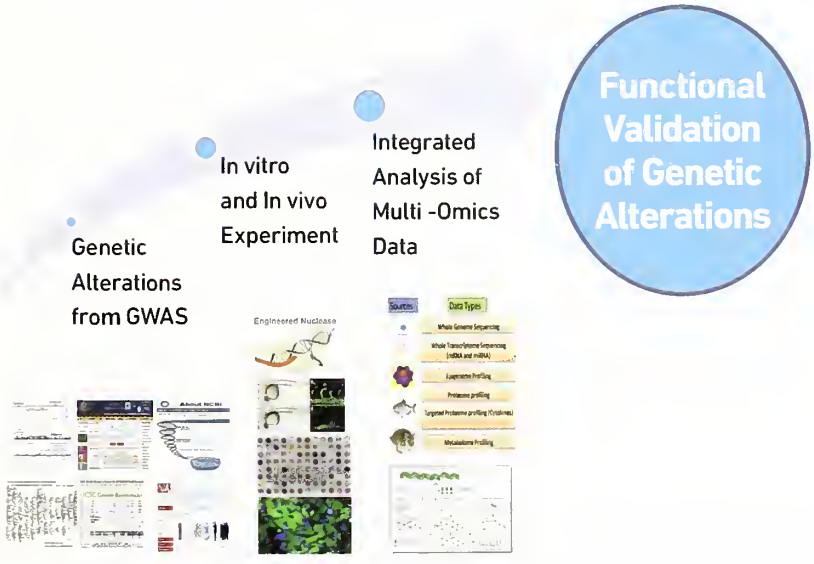


FIGURE 13. Functional studies of genetic variants associated with diseases and traits

Development of a prediction model based on missing heritability for complex diseases

Predictive disease models have progressed through a better understanding of the genetic role of a particular disease. Early findings of genetic variations are important in disease prevention because these variations could increase the risk for developing the disease. Additionally, family disease information is one of the most important factors to predict disease risk in this field, but is usually ignored. Here, we consider several statistical risk prediction models given an individual's genotype information and family history. Therefore, using data about genetic predisposition to a disease that incorporates family history can stratify people into various risk classes and might be meaningful for targeted screening. This approach could change lifestyle and precisely determine the treatment methods that may prevent the disease from developing, delay disease onset, or reduce the influence of the disease. The overall objective of our project was to screen the genetic effect of complex disease risk alleles at the population level and to conduct intensive follow-up of those with susceptibility to a genetic disease. Areas of research include (1) estimating various heritability traits to establish reference values for the Korean population using several statistical methods

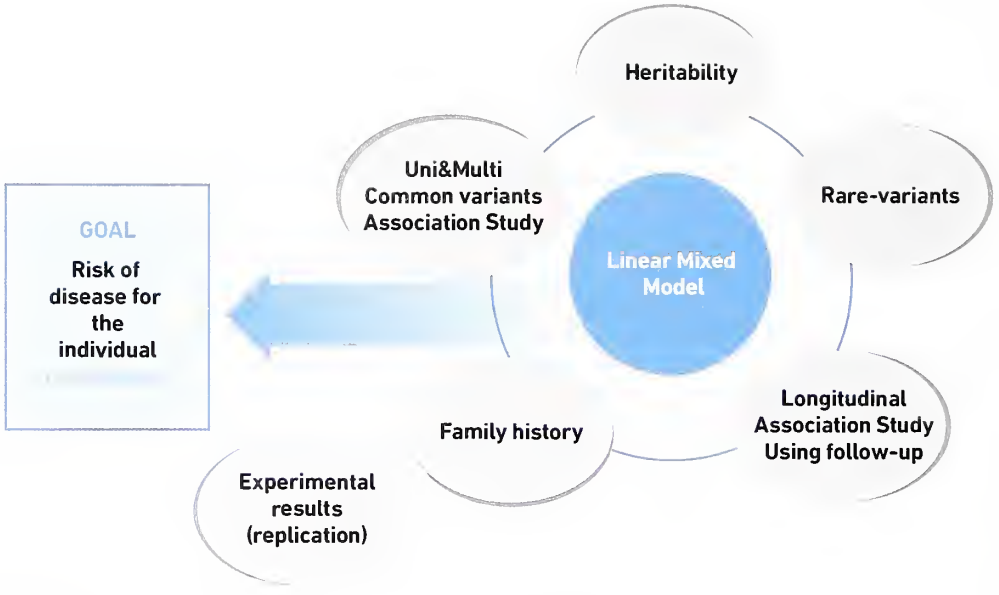


FIGURE 14. Functional studies of genetic variants associated with diseases and traits

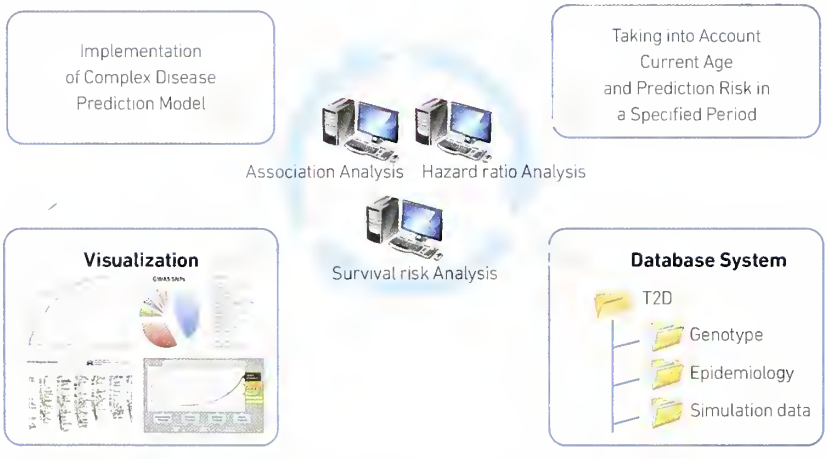


FIGURE 15. A public health surveillance system that considers both epidemiology and effect on genetic susceptibility to monitor risk factors for complex diseases.

(S.A.G.E and GCTA programs); (2) analyze multiple (-phenotypes or -SNPs) and repeated-measurement data genetic association studies to understand "missing heritability" in GWAS; and (3) develop a public health surveillance system considering both epidemiology and the effect on genetic susceptibility to monitor risk factors for complex diseases. We do not directly provide services, but conduct applied research that concentrates on enhancing research outcomes for clinical and public health interventions for all Koreans affected by complex diseases.

Korean Epigenome Project (KEP)

Korean Epigenome Project (KEP)

The KEP was launched in 2012 to produce 50 epigenomic sets on Korean chronic diseases related to target cells. The objective of the KEP was to provide a comprehensive epigenomic map of Korean chronic disease-related target tissues. Single cells were isolated from the pancreas, fat and kidney. Islet, ductal, acinar, and beta cells from the pancreas were purified into a single homogenous cell type using flow-assisted cell sorting. Adipocytes and pre-adipocytes were purified from fat, using Percoll gradient centrifugation. Mesangial, distal tubule, proximal tubule, podocytes, and collecting duct cells from the kidney were collected using

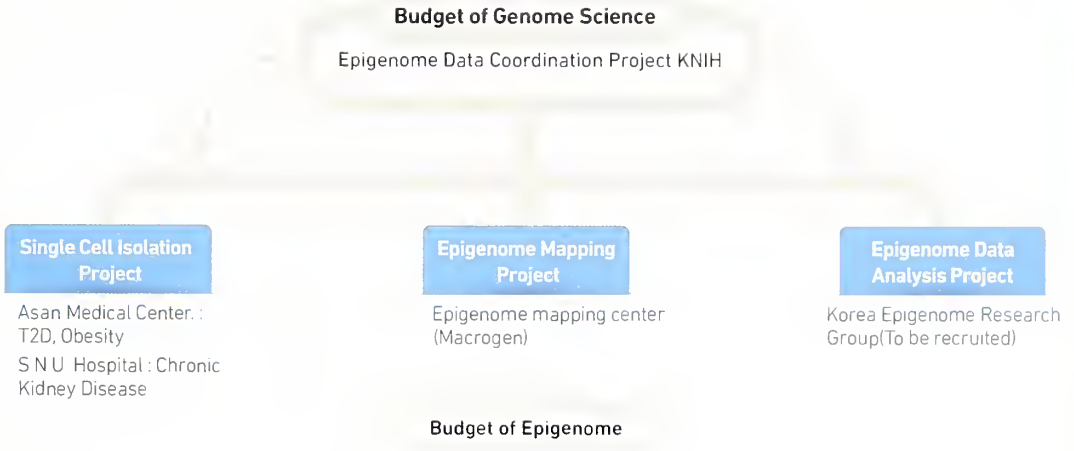


FIGURE 16. Structure of Korean epigenome project

mechanical sieving and immunostaining methods. Each cell type was derived from normal tissue removed by surgery. Patients were comprised of normal individuals and those with diabetes, obesity, or chronic kidney disease. Ten reference epigenome datasets of pancreatic and fat cells were produced. Cell types such as the islets, ductal, acinar, and beta from the pancreas and adipocytes, and preadipocytes from fat tissue were the primary target to produce a Korean metabolic reference epigenome. Bisulfite converted-whole genome sequencing, MBD-Seq, Infinium 450k DNA methylation bead array, open chromatin sequencing, and exome sequencing were performed to produce a comprehensive epigenomic map The standardization, quality control, and drawing of the epigenomic map from the epigenomic dataset will be performed next year.

The data will be deposited for public use by the International Human Epigenome Consortium

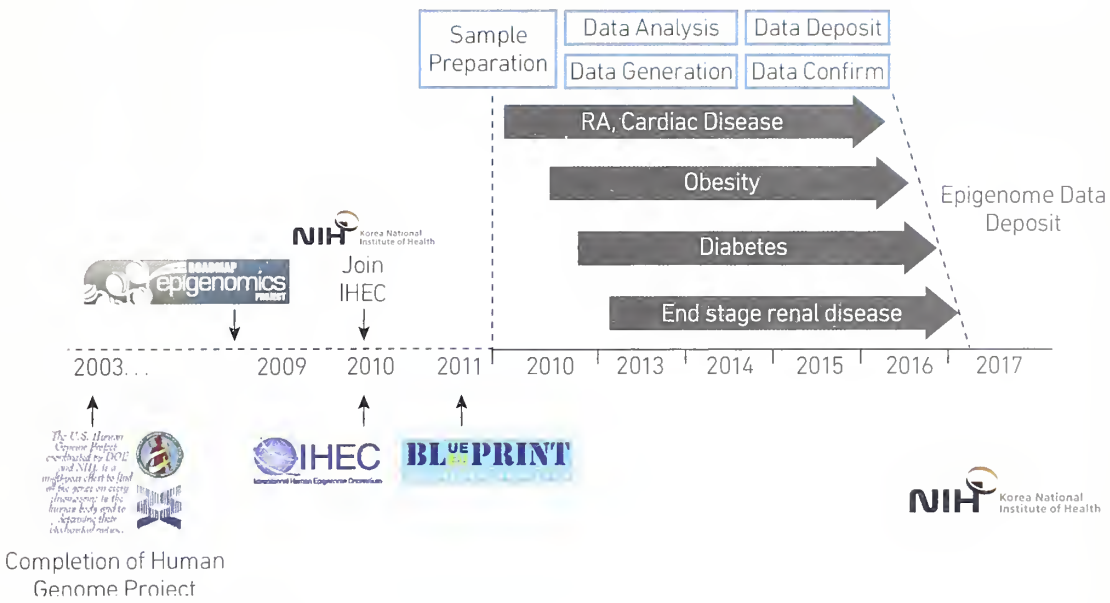


FIGURE 17. Long-term plan of the Korean epigenome project

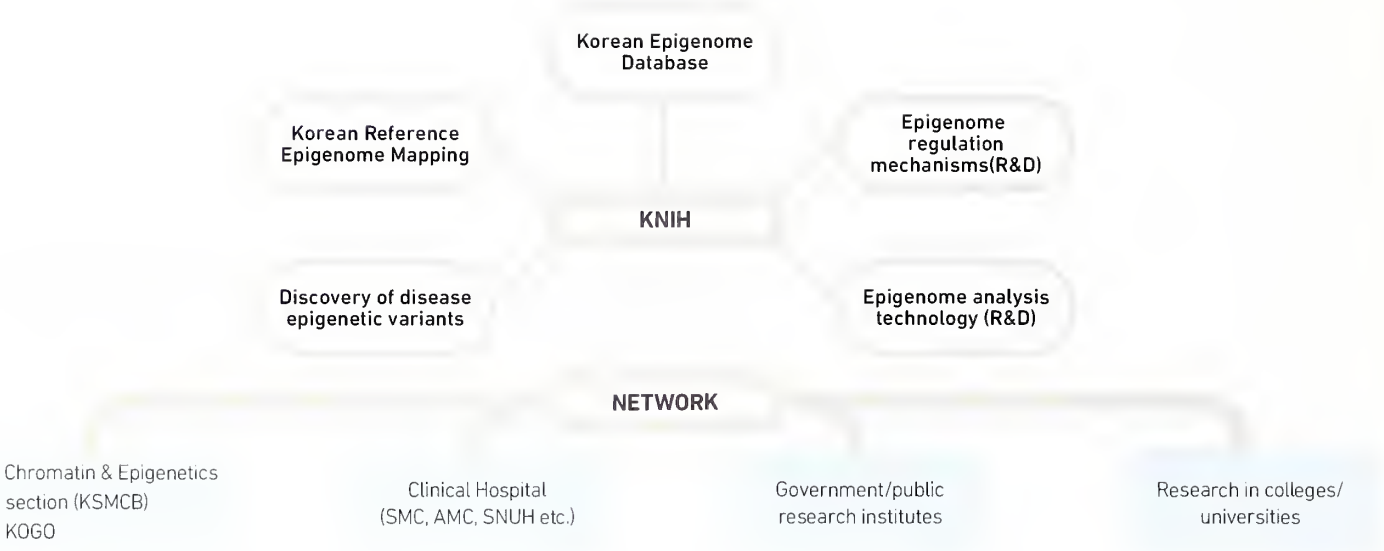


FIGURE 18. Collaboration network for Korean epigenomic research

(IHEC) Epigenome Track Hub. A workgroup on assay standards, metadata, data ecosystems, bioethics, communication, and cells and tissues was organized and is developing standard protocols for IHEC epigenome data production and collaboration. At the 2013 IHEC meeting in Berlin, the KNIH reported on current progress of the IHEC epigenome production plan and updated the disease epigenome analysis.

Collaboration: Data sharing and collaboration are underway with several prominent young investigator groups recruited from a Korea Genome Organization and Epigenome Society of the Korea Society of Molecular and Cellular Biology announcement. Data sharing and collaboration are open to any researcher who is interested in epigenomics research related to chronic human diseases.

KBP(Korea Biobank Project)

BIOBANK: AN ESSENTIAL TOOL FOR BIO-MEDICAL RESEARCH

Biobanks provide human biospecimens and personal clinical, epidemiological, and genetic data for biomedical researchers. These resources play vital roles in identifying disease risk factors, drug discovery, the development of diagnostic markers, and in the implementation of personalized medicine.

Personalized medicine is emerging as the main paradigm for next-generation medicine, as it provides tailored prevention and treatment of diseases for each person based on individual genetic background. A large amount of genetic data, produced from human biospecimens, is

required to pave the way for the development of personalized medicine, by providing information about genetic differences in races, tribes, and individuals. Furthermore, these data will facilitate the identification of individual disease risk factors including genetic, lifestyle, and environment factors.

Worldwide biobanks make an effort to develop bio-medical research and advance personalized medicine by collecting and distributing human biospecimens.

OVERVIEW OF THE KOREA BIOBANK PROJECT (KBP)

The Ministry of Health and Welfare in Korea launched the KBP in 2008 to enhance biomedical research. The Korea Biobank Network (KBN) was established to reach this goal and is composed of

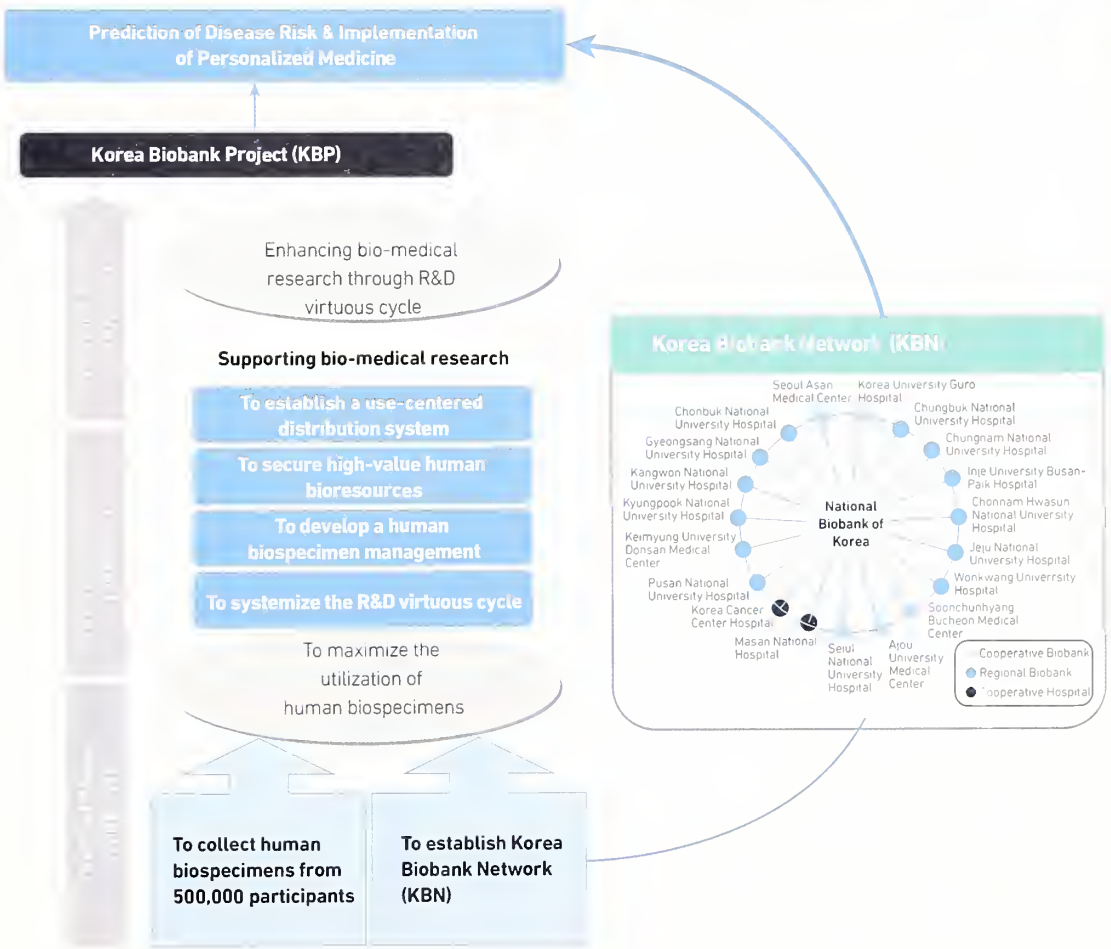


FIGURE 19. Introduction to the Kora Biobank Project (KBP)

the National Biobank of Korea (NBK) at the KNIH and 17 regional biobanks at university-affiliated hospitals.

The first phase (2008~2012) of the KBP aimed to collect human biospecimens from 500,000 participants to distribute human biospecimens for 500 research projects, and to be one of the top five worldwide biobanks. The NBK has collected cohort-based biospecimens through various cohorts and the Korea National Health and Nutrition Examination Survey. In addition, it has managed the regional biobanks and created a standardized management system for human biospecimens. The regional biobanks have collected disease-based biospecimens from patients who have visited their hospitals. Human biospecimens of 521,316 participants have been collected up to December 2012. These human biospecimens have been distributed for 657 research projects and 246 research studies have been published from results using KBN human biospecimens.

The second phase (2013~2015) of the KBP was started in 2013, with the objective of distributing human biospecimens to 1,000 research projects. During this period, the KBN will make an effort to establish a use-centered distribution system, to secure high-value bioresources, to develop a human biospecimen management system, and to systemize the R&D virtuous cycle.

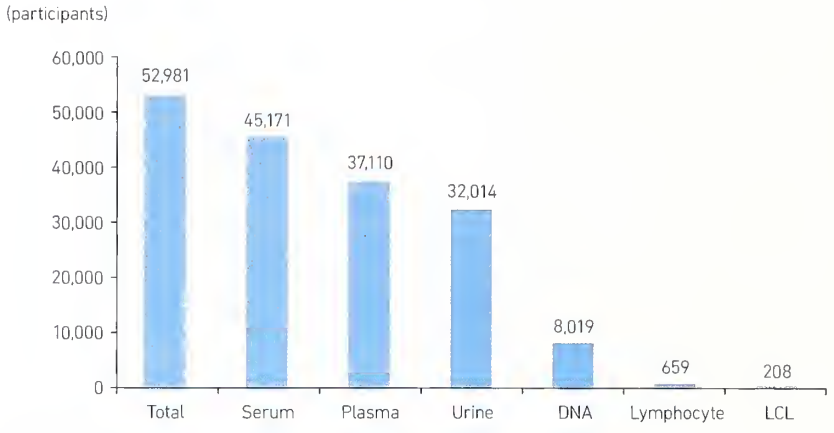


FIGURE 20. The status of human biospecimens collection in 2013

NBK: 2013 PERFORMANCE

The NBK collected cohort-based biospecimens from 52,981 participants in 2013, including serum (45,171), plasma (37,110), urine (32,014), DNA (8,019), lymphocytes (659), and lymphoblastoid cell lines (LCL, 208).

A total of 7,310,979 vials from 380,046 participants have been collected from various cohorts for the KoGES, disease prevention research, immunology and communicable diseases research, and genome and biomedical science research.

Of these, 25,452 vials of human biospecimens were distributed to 26 biomedical research projects in 2013. These included 4,450 serum, 15,040 DNA, 420 LCL, and 5,542 vials of LCL-DNA. A total of 258,303 vials were provided for 170 research projects up to 2013. Genetic and epidemiological information was provided for 27 and 32 research projects, respectively, in 2013. During the KBP period, these were provided for 90 and 93 research projects, respectively. In total, 165 research studies were published as of 2013.

The NBK published SOPs to systematize management of human biospecimens. This SOP contains collection, storage, and quality control guidelines according to the types of human biospecimens.

Furthermore, the NBK established the Distribu tion Desk of Korea Biobank Network and Biobank Information Management System (BIMS) ver. 3.0. The Desk of Korea Biobank Network is an online distribution system for user-centered distribution in which bio-medical researchers can search human

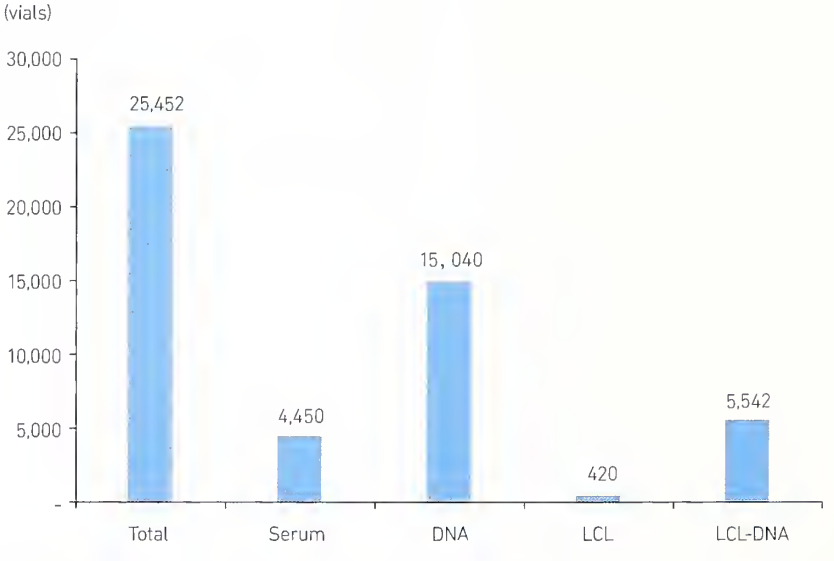


FIGURE 21. The status of human biospecimens distribution in 2013

TABLE 4. Total number of cohort participants, 2001–2013

Cohort		Accumulated Participants
Korean Genome and Epidemiology Study	Population-based Cohort	210,074
	Gene-Environmental Model Cohort	19,193
	Community-based Cohort	12,028
	Other Cohort	15,333
Subtotal		**256,628**
Disease Prevention Research	The Korea National Health & Nutrition Examination Study	63,142
	National Infectious Disease Serosurveillance Study	2,139
Subtotal		**65,281**
Immunology and Communicable Diseases Research	National Measles Serosurveillance Study	34,536
	National Brucellosis Survey	7,439
	AIDS Cohort	1,348
	Korea HPV Cohort Study	711
	Korea HCV Cohort Study	150
Subtotal		**44,184**
Genome and Biomedical Science Research	Major Birth Defects Cohort	3,854
	Geriatric Diseases Prevention and Management Cohort	2,025
	Polycystic Ovarian Syndrome Cohort) / Genome Research	2,000
	Korean Children-Adolescents Cohort Study	1,626
	Polycystic Ovarian Syndrome Cohort / Biomedical Science Research	1,251
	Korea Longitudinal Study of Aging	911
	Avellino Cohort	871
	Prevalence and Risk Factors of Pregnancy Complications	523
	Primary Care Family Cohort	514
	Study for Analyzing Korean Reference Genome	194
	A Demonstration Project for the Utilization of Secured Tissue-derived Resources	100
	Development of Paneled-Platforms of Rare Genetic Disorders based Human Specimens	64
	Prospective and Retrospective Analysis of Amyloidosis Patients : Early Diagnosis and Prognostic Factor of Amyloidosis	20
Subtotal		**13,953**
Total		**380,046**

TABLE 5. Total number of research projects performed using cohort-based biospecimens

Year	Human Biospecimens	Genetic Information	Epidemiological Information
2003	5	-	-
2004	9	-	-
2005	11	-	-
2006	11	-	-
2007	13	-	-
2008	18	8	9
2009	12	13	9
2010	18	8	8
2011	24	12	12
2012	23	22	23
2013	26	27	32
Total	**170**	**90**	**93**

biospecimen information and apply for distribution of human biospecimens and related information. BIMS ver. 3.0 is more flexible than the previous BIMS, as it allows for customization of necessary clinical information according to the disease and for long-term informational follow-up on patients.

REGIONAL BIOBANKS: 2013 PERFORMANCE

Seventeen regional biobanks collected disease-based biospecimens from 44,996 participants in 2013. These include plasma (34,697), serum (35,052), tissue (8,227), buffy coat (29,574), DNA

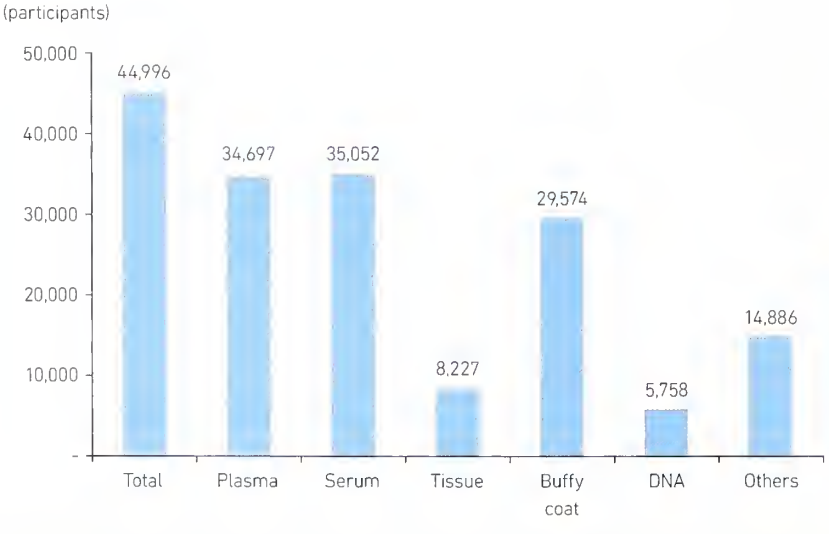


FIGURE 22. The status of human biospecimens collection in 2013

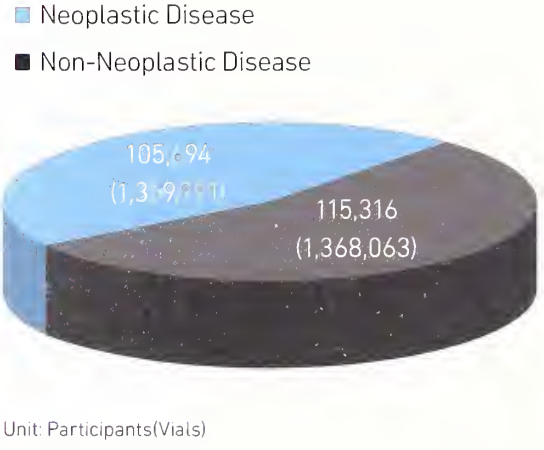


FIGURE 23. Collection Status of Disease-based biospecimens, 2008-2013

TABLE 6. Total number of disease-based participants, 2008–2013

Category	Disease	Accumulated Participants
Neoplastic Disease	Malignant neoplasm of digestive organs	39,973
	Malignant neoplasm of thyroid and other endocrine gland	15,613
	Malignant neoplasm of respiratory and intrathoracic organs	9,858
	Benign neoplamas	9,498
	Malignant neoplasm of breast	8,749
	Malignant neoplasm of urinary tract	3,598
	Malignant neoplasm of lymphoid, hematopoietic and related tissue	3,428
	Malignant neoplasm of female genital organs	3,341
	Neoplsasms of uncertain or unknown behavior	2,449
	Malignant neoplasm of male genital organs	2,400
	Diseases of the blood and blood-forming organs	2,296
	Malignant neoplasm of ill-defined secondary and unspecified sites	2,001
	In situ neoplamas	1,196
	Other neoplastic disease	1,294
Subtotal		105,694
Non-Neoplastic Disease	Diseases of the circulatory system	20,972
	Diseases of the respiratory system	14,011
	Symptoms, signs and abnormal clinical and laboratory findings	13,347
	Diseases of the digestive system	12,959
	Diseases of the genitourinary system	10,235
	Certain infections and parasitic diseases	9,684
	Diseases of the musculoskeletal system and connective tissue	6,368
	Factors influencing health status and contact with health services	6,001
	Endocrine nutritional and metabolic diseases	4,824
	Mental and behavioral disorders	4,183
	Certain conditions originating in the perinatal period	3,884
	Diseases of the nervous system	2,077
	Diseases of the eye and adnexa	1,826
	Diseases of the skin and subcutaneous tissue	1,812
	Congenital malformations, deformations and chromosomal abnormalities	1,087
	Injury, poisoning and certain other consequences of external causes	1,069
	Other non-neoplastic disease	977
Subtotal		115,316
Total		221,010

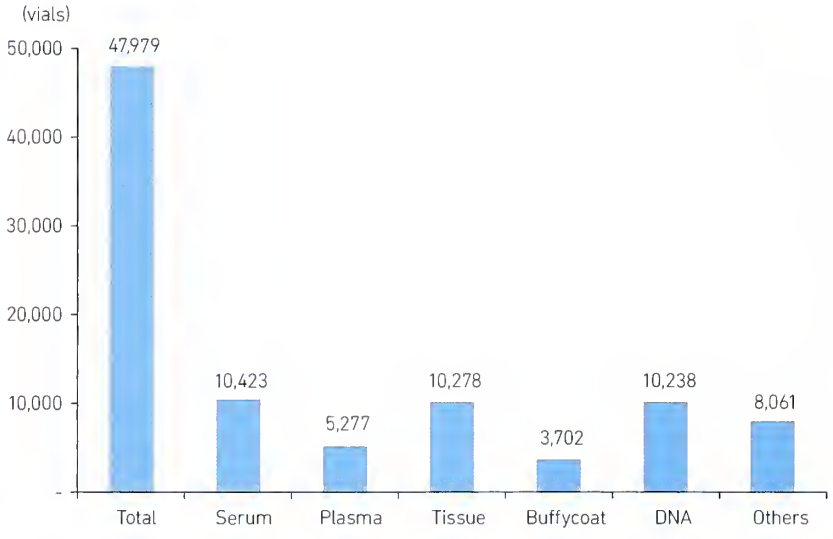


FIGURE 24. The status of human biospecimens distribution in 2013

(5,758), and other biospecimens (14,886). A total of 2,728,054 vials of human biospecimens from 221,010 participants were collected from 2008 to 2013. There are 1,359,991 vials from 105,694 participants with neoplastic diseases and 1,368,063 vials from 115,316 participants with non-neoplastic diseases.

Among them, 47,979 vials of human biospecimens were distributed for 209 bio-medical research projects during 2013 and 159,416 vials were distributed for 728 research projects from 2008 to 2013. As a result, 200 research studies have been published as of 2013.

TABLE 7. Total number of research projects performed using disease-based biospecimens

Year	No. of Biobanks	No. of Research Projects
2008	8	27
2009	12	12
2010	13	18
2011	17	24
2012	17	23
2013	17	26
Total		170

FUTURE PLANS

During the second phase of the KBP, the NBK will secure and distribute high-value human biospecimens to promote public health by enhancing bio-medical research. Furthermore, the NBK will develop R & D projects for collection and utilization of human biospecimens with clinical and genetic data. These resources will play an important role in the construction of an R & D virtuous cycle system. Each of the 17 regional biobanks will collect specialized disease-based human biospecimens and related information.

Accomplishments

Publications

1. Hong KW et al. Replication of genomewide association studies on age at menarche in the Korean population. Genes & Genomics. 35(1):69-75 (2013)
2. Hong KW et al. Genome-wide association study of serum albumin:globulin ratio in Korean populations. Journal of Human Genetics. 58(3):174–77 (2013)
3. Hong KW et al. Genome-Wide Association Study of Orthostatic Hypotension and Supine-Standing Blood Pressure Changes in Two Korean Populations. Genomics Inform. 11(3):129-34 (2013)
4. Kim YJ et al. Genome-wide association study meta-analysis reveals transethnic replication of mean arterial and pulse pressure loci. Hypertension. 62(5):853-859 (2013)
5. Ma RC et al. Genome-wide association study in a Chinese population identifies susceptibility locus for type 2 diabetes at 7q32 near PAX4. Diabetologia. 56(6):1291-1305 (2013)
6. Hwang JY et al. Meta-analysis identifies a MECOM gene as a novel predisposing factor of osteoporotic fracture. Journal of Medical Genetics. 50(4):212-219 (2013)
7. Moon S et al. KGVDB: a population-based genomic map of CNVs tagged by SNPs in Koreans. Bioinformatics. 29(11):1481-1483 (2013)
8. Hwang JY et al. Recapitulation of previous genome-wide association studies with two distinct pathophysiological entities of gastric cancer in the Korean population. Journal of Human Genetics. 58(4):233-234 (2013)
9. Lee JY et al. A genome-wide association study of a coronary artery disease risk variant. Journal of Human Genetics. 58(3):120-126 (2013)
10. Go MJ et al. New susceptibility loci in MYL2, C12orf51 and OAS1 associated with 1-h plasma glucose as predisposing risk factors for type 2 diabetes in the Korean population. Journal of Human Genetics. 58(6):362-365 (2013)
11. Kim YK et al. Gene-based copy number variation study reveals a microdeletion at 12q24 that influences height in the Korean population. Genomics. 101(2):134-138 (2013)
12. Kim YU et al. EvoSNP-DB: A database of genetic diversity in East Asian populations. BMB Reports. 46(8):416-421 (2013)
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16. Bae J et al. Perspectives of International Human Epigenome Consortium. Genome & Informatics. 11(1):7-14 (2013)
17. Bae J et al. Perspectives of International Human Epigenome Consortium. Genome & Informatics. 11(1):7-14 Jadamba E et al., A pruning strategy of reference panels for fast SNP genotype imputation. BioChip Journal. 7(1):6-10 (2013)
18. Kim J et al., A block-based imputation approach with adaptive LD blocks for fast genotype imputation. BioChip Journal. 7(1):63-67 (2013)
19. Heo HS et al., A nutrigenomic framework to identify time-resolving responses of hepatic genes in diet-induced obese mice. Molecules and Cells. 36(1):25-35 (2013)
20. Kim YU et al., EvoSNP-DB: A database of genetic diversity in East Asian populations. BMB Reports. 46(8):416-421 (2013)
21. Shim SM, Jung SY et al. Network signatures of cellular immortalization in human lymphoblastoid cell lines. BBRC. 441: 438-446(2013)
22. Lee JE et al. Instability at Short Tandem Repeats in Lymphoblastoid Cell Lines. Osong Public Health Res Perspect. 4(4)194-196 (2013)

Patents

23. Go MJ et al. Method of providing the information of single nucleotide polymorphism associated with low density lipoprotein. 10-2013-0121369.
24. Go MJ et al. Method of providing the information of single nucleotide polymorphism associated with fasting plasma glucose 10-1293745-0000.
25. Go MJ et al. Method of providing the information of single nucleotide polymorphism associated with renal function related trait. 10-1293746-0000.

Division of Planning and Research

The Division of Planning and Research aims to develop public benefit technologies that are necessary for examination, surveillance, prevention, diagnosis and treatment of infectious and chronic diseases. We have responded to challenges in order to create the healthier society, and established systems of the national disease management and the response process by selecting diseases with the purpose of preventing risks in the future. The planning among our activities is to maximize the use of research results with the efficient systems. So as to support strategic R&D plans for keeping the pace with the globalization of health and medical technologies, we endeavor to transfer technologies achieving outcomes of the R&D researches and build up the multiple collaborations with domestic and international organizations. [Fig 1]

PLANNING OF NATIONAL R&D PROJECTS

A National R&D projects[11] conducted by the KNIH have been planned and studied in order to use as a base of policies for the public health of people. Recently, the necessity of studying those fields has been focused because of increasing outbreaks of diseases like Avian Influenza(AI) and damages out of climate change. Getting ahead of the trend, KNIH has established long term chevelopment implementation plan reflected paradigm shift of health care technologies and infectious disease studies. The plan includes strategies for achieving the missions: response to infectious disease public health crises, development of chronic disease management technology and establishment of public health research infrastructures, etc.

KNIH promotes systemic and specific strategies concerning national R&D projects for planning research to achieve effectively a purpose. In 2013, KNIH conducted development researches to infectious disease management technology, chronic disease management technology, Korean genome analysis, convergence technology based on women health, operation of national stem cell bank and establishment of standards, and national stem cell regenerative research center and newly planned and studies 'acute and chronic diseases caused by climate change' to monitor and control infectious disease related to climate change. The division of planning and research are operating and managing all procedures of planning the national R&D projects in the KNIH.

1) National R&D projects are research and development projects in science and technology area conducted by the central administrative agency in accordance with the'Framework Act on Science and Technology'

IMPROVEMENT OF RESEARCH TASK MANAGEMENT AND RESULT MANAGEMENT SYSTEM

KNIH has determined strategies and core research fields regarding the institute's mission and long term development implementation plan, and performed relevant R&D projects in each field of diagnosis, prevent, surveillance, treatment, vaccine development and infrastructure. R&D research projects consist of 106 projects that are conducted by the institute itself and 34 projects that are commissioned to or conducted with other institutes.

According to the Act of Evaluation and Performance Management of the National R&D Projects, the 'Ministry of Science, ICT and Future Planning' evaluated the projects performed by KNIH as 'nomal' level in 2013.

Research outcomes from national R&D projects in 2013 are 46 research papers, 13 patent application, 18 patent registration, 2 technology transfer, building Korean genome information of about 60,000 people, Korean specific new marker development, bio-resources and information construction, use of dielectric information and database construction of chronic disease research information.

Meanwhile, 'Korea Centers for Disease Control and Prevention Research & Development Regulation' has been revised for efficient management of National R&D. The overall management system has been reorganized including planning, selection, process control, result evaluation and achievement management of research tasks and academic research services. In order to manage and utilize information and data collected from research tasks more efficiently, laboratory notebook has been revised. For the sake of the promotion of making public and use of research findings, KNIH gives a service to Korea Institute of Science and Technology Information following the related Act.

Henceforth, for the efficient management

of National R&D and maximization of research result application, it is planning to run a research group project that is focused on connecting and applying results. Also, to improve the specialty and competence of the National R&D management, it will perform researcher training. Regulations and guidelines will be revised according to the revision of law regarding National R&D and research result management system will also be reorganized.

STRENGTHENING DOMESTIC AND INTERNATIONAL RESEARCH COOPERATION SYSTEM

For the improvement of research competence and construction of preemptive disease control system, the need for intimate relationship and research cooperation between domestic and international organizations is increasing. KNIH also strengthened the collaborative researches in order to improve research development capability and research efficiency.

The division of Planning and Research will strive and support to build up the international collaborative research system as well as resource exchange and collaborative application system consistently. Not only that, research plans will be pushed forward according to the MOU agreed with international institutes and interchange human resources in the fields of public health to develop skilled scientists.

Division of Biosafety Evaluation and Control

A national biosafety management is designed to prevent infectious disease among personnel and to protect the community from harm by preventing the release of pathogens. The Division of Biosafety Evaluation and Control (DBEC) performs an effective biosafety management based on the 'Infectious Diseases Control and Prevention Act (No. 9932) and 'Act on Transboundary Movement etc. of Living Modified Organism(LMO's)' and in South Korea.

For this purpose, the DBEC increases the regulatory compliance through a combination of training, inspections, evaluations, review and clear communication. We provide the guidelines of the biosafety and biosecurity, and support technologies for the construction and operation of containment facilities. We also develop programs related to biosafety and biosecurity with the purpose of enhancing capacities and carrying out policies and regulations established by the Ministry of Health and Welfare.

ENFORCEMENT OF NATIONAL BIOSAFETY EVALUATION AND MANAGEMENT

The outbreaks of the highly pathogenic avian influenza (H7N9), along with international concern regarding the use of modern biotechnology and the increasing use of human infectious agents in biomedical laboratories, have created the need for a national and international regulatory framework for biosafety.

We have two objectives. First, we establish and implement the national biosafety and biosecurity management systems for HDPs to enhance institutional capacities for the biosafety in biomedical research laboratories and to protect public health against the infectious materials. Second, we strengthen national biosafety supervision capabilities and develop national standard biosafety guidelines with the purpose of reducing biorisk.

National measures regarding the biosafety issues of Highly Dangerous Pathogens (HDPs)

As the subclauses of the 'Infectious Disease Control and Prevention Act' were amended in 2013, the highly pathogenic avian influenza (H7N9) virus was added in the 35 pathogens as HDPs which have potential have to pose a serious threat to public health and might be used as bioterriosm agent.

There were four notifications of the possession and one permit application from abroad in the highly pathogenic avian influenza (H7N9) virus. In addition, there were 6 cases in permit application from abroad and the notifications of 9 cases in the isolation, 6 cases in the transfer, and 10 cases in the possession.

The DBEC carried out the field inspections on the biosafety and biosecurity management in the 44 institutions of the HDPs possession and handling. As a result, in institutes that possess and handle HDPs, the percentage of the safety management compliance was above 95%. To provide information of biosafety and biosecurity and training opportunities, the DBEC hold the workshop for the management of HDPs and the 1st Korean Biosafety Conference for the Biosafety and Biosecurity Management, especially, ran pre-conference courses for the in-depth training of the attendees. In addition, the DBEC made kinds of publicity leaflets to enhance the awareness of the biosafety and biosecurity as well as material safety data sheet on the 35 HDPs

To boost of morale of the individuals and institutions which do handling and preservation of the HDPs and run the containments in the appropriate manner, Ministry of Health and Walfare awarded them.

National approval of the experiments involving recombinant DNA molecules

The national approval system has been initiated to monitor research studies related to Living Modified Organisms (LMOs) including unknown pathogens, introduction of drug-resistant genes, expression of toxins and the 35 HDPs in South Korea. There were two cases in the permit application of LMOs from abroad. A total of 48 study projects were

TABLE 1. The number of permit application and notification cases in 2013

No. of Permit application case	No. of Notification case		
	Isolation	Transfer	Possession
6	9	6	10

approved in Korea, 68.8% (n = 33) of them being run by national institutes. Among 28 research projects which approved during 2011-2013, there were 32% (n = 9) and 18% (n = 5), 43% (n = 12) focusing on human-derived influenza virus and a drug-resistant gene and HDPs, respectively. In terms of biosafety and biosecurity, 24 events were carried out inspection by self or national officers in 2013. To control developed LMO under national approval system, annual inspections will be carry out

RISK ASSESSMENT OF LMOs FOR HUMAN HEALTH

A risk assessment is a process intended to calculate or estimate the risk to a given target organism, system, or (sub)population, including the identification of uncertainties, following exposure to a particular agent, taking into account the inherent characteristics of the agent of concern, as well as the characteristics of the specific target system. The objective of a risk assessment under 'Act on Transboundary Movements etc. of LMOs' and 'The Cartagena Protocol on Biosafety' is to identify and evaluate the potential adverse effects of LMOs on the conservation and sustainable use of biological diversity in the likely potential receiving environment, taking also into account risks to human health'

We have two main objectives. KCDC was performed to ensure public health from potential risks, and to support the advanced national biosafety infrastructure of the human risk assessment of LMOs. All human activities associate with carrying a risk through LMO exposure in KCDC during 2013 were agricultural use(for feed grains or flowering grass). In 2013, KCDC completed the risk evaluation of 4 events, and new 3 events are being processed.

In 2014, we will perform to pursue international harmonization of the risk assessment framework, so construct risk management system of LMO for public health and medical usage in Ministry of health and welfare. And we will perform to provide risk assessment & management guidelines of LMO for public health and medical usage, and provide risk assessment & management guidelines of Living modified microorganisms controlled Ministry of health and welfare.

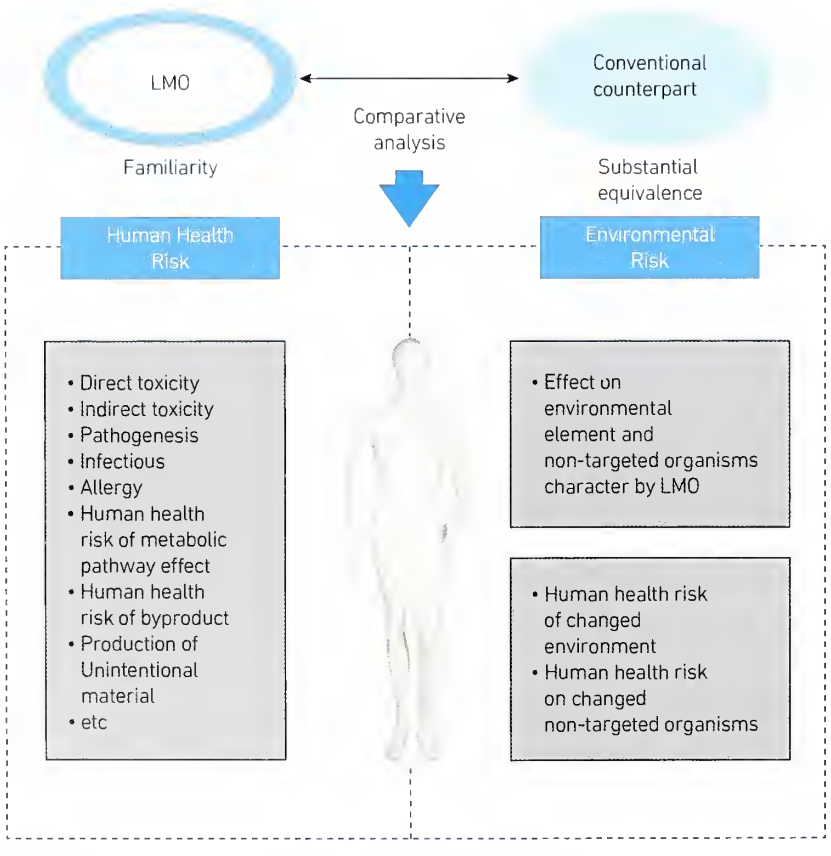


FIGURE 1. Risk assessment framework of LMO in Korea

THE NATIONAL MANAGEMENT OF CONTAINMENT LABORATORIES IN KOREA

The biosafety practices in bio-medical facilities have become more important because of emerging and re-emerging of pathogens and new strains with modern biotechnology. In order to enhance the biosafety capacity of buildings in Korea, the national approval and management of containment facilities, such as BL3 and BL4 facilities, has been developed with the 'Act on Transboundary Movements etc. of LMOs'.

To increase the biosafety awareness of BL3 facility managers, we inspected and ranked their management processes annually as having 85.1% compliance in 2013. To increase the biocontainment concept, we published transition index for Biological Containment facility of Biosafety Level 4 (BL4) and

TABLE 2. The number of approved BL3 facility

Year	Institutional type			Re-Approved No.
	University	Private Institution	National Institution	
2012	0	0	12	4
2013	0	0	3	3
Total	0	0	15	7

Animal Biosafety Level 3 (ABL3). Based upon theses guides, we hope it is helpful to make concept and design for biocontainment facility.

Based upon the revise of the 'Act on Trans-boundary Movements etc. of Living LMOs' in 2013, we will revise the notification of LMO's Act including laboratory accreditation, remodeling, and shutdown procedures. In this LMO guideline, we will define biosafety officer including qualification of academic background and training. Based upon the BL3 and ABL3 transition, we will develop the criteria and methods for validation of maximum containment (BL4) & detailed animal containment (ABL3) facility.

STANDARDIZATION OF THE PUBLIC HEALTH LABORATORY

To ensure an accurate diagnosis and identify disease factors, we will try to set up the standardization of the public health laboratory including quality assurance.

To standardize diagnosis and establish credibility for our test results, we obtained the ISO 17025 accreditation. For maintaining the ISO 17025, we carried out internal audits and reviewed of our management system. To extend our accreditation scope, we prepared documents and proficiency assurance results of two divisions, the Division of Biobank for Health Sciences and the task force team for Pathogen Resources. However, we cannot finish theses extend procedure but prepare new program for laboratory quality management system in Public Health sector.

For the quality control of each laboratory, the External Quality Assessment Schemes (EQASs) periodically operate for laboratory's testing process at individual testing sites, including hospitals, Public Health Centers (PHCs), and local Institutes of Public Health and the Environment (LIHE). In 2013, we organized HIV and Syphilis Proficiency test programs for both public and private sectors. In HIV Proficiency test program operated twice at May and Nov. Each operation attended about more than 500 laboratories of each scheme. Syphilis scheme was operated in Nov for 270 laboratories include pubic health centers and some private hospitals.

To achieve accreditation as a Certified Reference Materials (CRM) producer, the KCDC finalized quality management documents and constructed a production schedule following ISO Guide 34 and 35. Following these documents, the KCDC plans to produce the CRM in 2015. In 2013, we tried to produce HIV antiserum with different titers and Human & Bacterial DNA. After homogeneity testing, we tested stability for short and long term periods.

To make new quality management systems of communicable disease diagnosis laboratory, we will try to revise 'Act on communicable disease control and prevention' including laboratory quality management system, quality assurance tools, and human resources. In addition to setup of standardization for communicable disease diagnosis laboratory, we also extend this kind of quality management system to general health laboratory with revise 'Framework Act on Health and Medical Services'

THE MANAGEMENT OF LABORATORY ANIMAL FACILITY

Then laboratory animal facility was founded to assist the execution of animal research (infectious disease, vaccines, chronic disease research, etc.), securing

reliance and accuracy of research results, and supporting animal research and educating human resources through professional knowledge about animal research, following the law of laboratory animal (2010). we have provided a portfolio of laboratory animal research and commercial services 'The animal care and use program' to researchers in KNIH, Since 2011.

We have carried out the following tasks in 2013, the supporting services were performed for using an average of 3,718 laboratory animals each month, we have conducted the business in the cleansing and sterilization of the breeding apparatuses, disposal of the dead bodies and medical waste, and the construction of warning systems for rodents IVC (individual ventilated) cages. Our team published The Guideline of Animal Care and Use Program in KCDC, and the 27 SOPs were issued for the use and management of the laboratory animals. Our facility supplied the anesthetic agents such as Ketamin, Barbiturates since July, 2013, that is used to put animal under anesthesia for the euthanasia of laboratory animals. We have monthly educated the researcher for the use and management of animals. Also, the quarantine system was introduced in order to provide healthy laboratory animals in consideration of veterinary management.

Our 2014 business plans are as follows: 1. Improvement of veterinary care, 2. Integrative management of laboratory animal supply for the reasonable use, 3. Maintaining a clean environment for laboratory animal breeding, 4. Enactment of the guide book and SOP for the use and management of laboratory animals. 5. Experiment technique training program will be held on that can minimize the stress and pain of laboratory animals. 6. Establishment of laboratory animal health monitoring system for usage of healthy animals, 7. Post managements for the approved animal experiment protocol, we will investigate the actual conditions of the animal experiment compared to the approved protocol.

LABORATORY SAFETY MANAGEMENT

The laboratory safety aims to minimize the risk of injury or illness to laboratory workers by ensuring that they have the correct training, information and support. To prevent researchers from safety-related accidents in laboratory, DBEC carried out a laboratory safety education and inspection according to the laws and regulations related with laboratory safe environment development. In 2013, 256 (93.4%) persons completed the regular course of laboratory biosafety education in the first half of the year and 255 (96.2%) persons did in the second half of the year. Newcomers learned laboratory biosafety education including individual safety equipment, chemicals and waste disposal in every month. Moreover, all laboratories of KNIH were inspected quarterly for the microbial experiments, fire hazard issues, electrical equipment, gases, chemicals, medical waste, individual safety equipment and other machineries. In 2013, most (97%) of KNIH laboratory graded 1 and 2 from the precision safety diagnosis. DBEC published laboratory safety manual, medical waste manual and 2012 KCDC white paper on laboratory safety.

In 2014, DBEC make plans for the laboratory safety management as follows. The quarterly inspection and semiannually education of the regular institutional laboratory biosafety will be carried out. Newcomers will be provided with safety information by laboratory safety training for 8 hours in every month. The vaccinations for dangerous communicative diseases by hantavirus, influenza virus and some other pathogens will be administered to researchers. To establish chemical laboratory safety systems, DBEC will provide ventilation chemical cabinet and security goods, and carry out the special health medical examination.

Accomplishments

Productions and Publications:

1. Biosafety Newsletters, 2013 (Mar, Jun, Sep, Dec)
2. Guide for Laboratory Animals Care and Use in KCDC, 2013
3. Guidance for Transport of Pathogens and Infectious substances, 2013
4. Guide to safety transport of infectious substances, 2013
5. Highly Dangerous Pathogens biosafety data sheets, 2013
6. Laboratory safety manual in KCDC, 2013
7. Medical waste manual in KCDC, 2013
8. Notification of construction and operation for Institutional Biosafety Committee, 2013
9. 2012 KCDC white paper on laboratory safety, 2013
10. Index for Biological Containment Facility of Animal Biosafety Level 3 (ABL3)
11. Index for Biological Containment Facility of Biosafety Level 3 (BL3)
12. Index for Biological Containment Facility of Biosafety Level 4 (BL4)

Event and Education:

13. Workshop for Management of Highly Dangerous Pathogens, 2013 (Jun 20 to 21), Glory condo, Busan, 78 attendees
14. Seminar for statistical method of testing validation (Oct 14 ~ 15), 29 attendees from 13 divisions, KCDC
15. The 1st Annual Korean Biosafety Conference, 2013(Oct 31 to Nov 1), Daemyung resort, Jeonnam, 217 attendees
16. Seminar for uncertainty of testing in health laboratory (Dec 10), 8 attendees from 3 division, KCDC
17. Regular: Laboratory Biosafety Inspection of the KCDC, 2013 (Mar, Jun, Sep, Dec)
18. Education and Training for Laboratory Biosafety Management, 2013 (Jun, Oct to Dec)
19. Workshop for Management of BL3 Laboratories 2013 (Jan 2 to Feb 1), Yongpyong resort, gang won, 35 attendees

Symposia & Conferences

MOU signed between the Korea National Institute of Health and Osong Medical Innovation Foundation

January 29, 2013
Osong Medical Innovation Foundation

KNIH and Osong Medical Innovation Foundation agreed to collaborate in facilities, equipment human resources andinformation exchanges etc.

MOU signed between the Korea National Institute of Health and the Pasteur Institute

April 8, 2013
National Biobank of Korea

KNIH and Institut Pasteur Korea signed a memorandum of understanding to strengthen national health research and development competitiveness and to establish a framework of innovative drug discovery technology based on the bio-imaging technology.

The 12th Osong International Bio-Symposium

September 11, 2013
Exhibition Hall II, KINTAX, Seoul

The purpose of this symposium was to discuss current KNIH activities for biobanking and stem cell research fields. The symposium was comprised of two sessions, International Biobank Standardization and Pluripotent Stem Cell and Bioinformatics.

The 2nd KNIH-KSOG Joint Symposium on Women's Health Research

September 27, 2013
Grand Hilton Hotel, Seoul

The purpose of this event was to discuss recent trends and key issues of women's health, and to share the information of the national plan of women's health research and women's health statistics in Korea. The subjects of the session were 'Menopause' and 'Preterm birth'.

The 1st Annual Korean Biosafety Conference

October 31~ December 1, 2013
Daemyung resort, Jeonnam

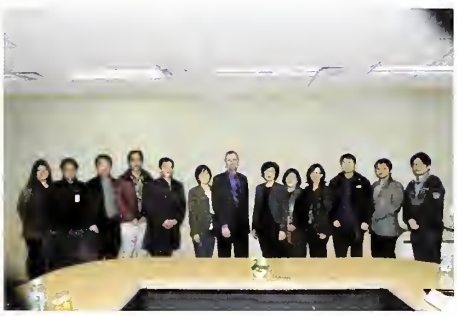
The 1st Annual Korean Biosafety Conference' was held to provide information of biosafety and bio-security and offer training opportunities including two pre-conference courses for risk assessment and BL3 facility management.

IHEC Annual Meeting

November 10-12, 2013
Berlin, Germany

The KNIH delegates (The Center for Genome Science) attended at the IHEC 2013 Berlin Meeting held in the historical "Kaiserin Friedrich-Haus", located in the heart of Berlin on the famous Charite campus. About 200 IHEC scientists and funding agency members from around the globe were attended

Meeting with NHGRI director



November 12, 2013
Seminar room, KNIH

Dr. Eric Green visited KNIH on November 12, 2013. The meeting gave a valuable chance to expand his understanding about KNIH and KFDA activities and the current research activities on the human genome in the Center for Genome Science. The meeting made a close relation between KNIH and NHGRI for future human genome research.

2013 NIH Annual Scientific Conference

December 17, 2013
National Biobank of Korea

'2013 NIH Annual Scientific Conference' was held to encourage the sharing of research achievements to maximize the research efficiency and to increase the researcher morale.

Publication Committee and Publication Information

Publication Information

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